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TOXICITY AND METABOLISM STUDIES WITH EPA PRIORITY POLLUTANTS AND RELATED CHEMICALS IN FRESHWATER ORGANISMS

by

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16. ABSTRACT

Toxicological studies were conducted in two areas: (1) the toxicity, bioconcentration potential and metabolism of five herbicides in fish; and (2) the toxicity and/or metabolism of priority pollutants and related chemicals in various aquatic organisms.

The test herbicides included alachlor [2-chloro-2',6'-diethyl-N-(methoxymethyl) acetanilide], bromacil (5-bromo-3-*sec*-butyl-6-methyluracil), dinoseb [2-(*sec*-butyl)-4,6-dinitrophenol], diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea], and propanil (3,4-dichloropropionanilide). Acute toxicity (through 192 hr), early life-stage toxicity (58-64 day), and bioconcentration studies were conducted with fathead minnows (*Pimephales promelas*) in Lake Superior water. Herbicide metabolism was investigated in rainbow trout (*Salmo gairdneri*) both *in vivo* and *in vitro*.

Twenty-two chemicals from the EPA priority pollutant list were studied for their acute and/or chronic toxicity to selected freshwater organisms. These included 1,2-dichloroethane, 1,1,2-trichloroethane, 1,1,2,2-tetrachloroethane, tetrachloroethylene, 1,2-dichlorobenzene, 1,3-dichlorobenzene, 1,4-dichlorobenzene, hexachlorobenzene, hexachlorobutadiene, di-*n*-butylphthalate, pentachlorophenol, heptachlor, chlordane, toxaphene, arsenic^{+3}, chromium^{+6}, lead^{+2}, mercury^{+2}, nickel^{+2}, silver^{+1}, selenium^{+4}, and cyanide. Freshwater species tested included the fathead minnow, rainbow trout, bluegill sunfish (*Lepomis macrochirus*), flagfish (*Jordanella floridae*), *Daphnia magna*, scud (*Gammarus pseudolimnaeus*), midge (*Tanytarsus dissimilis*) and green alga (*Selenastrum capricornutum*). Toxicity tests were also conducted with pentachloroethane, hexachloroethane, 1,2,4-trichlorobenzene, pentachlorobenzene, methanol and dimethylformamide. The uptake by fish of di-*n*-butylphthalate from water, its metabolism and elimination were investigated. Comparative metabolism of 1,1,2-trichloroethane, chlorobenzene, 1,1,2-trichloroethylene, chloroform, and carbon tetrachloride was studied in rainbow trout and *Daphnia*.

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ABSTRACT

Twenty-two chemicals from the EPA priority pollutant list were studied for their acute and/or chronic toxicity to selected freshwater organisms. These included 1,2-dichloroethane, 1,1,2-trichloroethane, 1,1,2,2-tetrachloroethane, tetrachloroethylene, 1,2-dichlorobenzene, 1,3-dichlorobenzene, 1,4-dichlorobenzene, hexachlorobenzene, hexachlorobutadiene, di-*n*-butylphthalate, pentachlorophenol, heptachlor, chlordane, toxaphene, arsenic^{+3}, chromium^{+6}, lead^{+2}, mercury^{+2}, nickel^{+2}, silver^{+1}, selenium^{+4}, and cyanide. Freshwater species tested included the fathead minnow (*Pimephales promelas*), rainbow trout (*Salmo gairdneri*), bluegill sunfish (*Lepomis macrochirus*), flagfish (*Jordanella floridae*), water flea (*Daphnia magna*), scud (*Gammarus pseudolimnaeus*), midge (*Tanytarsus dissimilis*), and green alga (*Selenastrum capricornutum*).

Toxicity tests were also conducted with pentachloroethane, hexachloroethane, 1,2,4-trichlorobenzene, pentachlorobenzene, dimethylformamide and methanol. Di-*n*-butylphthalate uptake from water, elimination and metabolism by fish was studied. A comparison was made of the metabolism and binding of carbon tetrachloride, chloroform, 1,1,2-trichloroethane, 1,1,2-trichloroethylene and monochlorobenzene by microsomal fractions of rainbow trout livers and of daphnid whole bodies.

FOREWORD

The Environmental Research Laboratory-Duluth is concerned with effects of chemical pollutants upon aquatic life. Many chemicals are presently in use without adequate knowledge of their effects on aquatic life, and new chemicals are continuously being developed and marketed.

This report contains information on thirty-two chemicals and their effects on freshwater life. Included are values for acute toxicity, chronic toxicity, and metabolism of these chemicals with various species of organisms. These values can be used to provide guidance for the protection of aquatic life.

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Director
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SECTION I

INTRODUCTION

A 1978 court settlement referred to as the "EPA Consent Decree" between EPA and several environmentally concerned organizations as plaintiffs resulted in the publication of a list of toxic pollutants for which effluent limitations and guidelines were to be developed (Keith and Telliard, 1979). This list of "priority pollutants" initially consisted of 65 chemicals (or groups of chemicals), and was later expanded to 129 entries. EPA was charged with the responsibility of determining the hazard potentials of these "priority pollutants" to aquatic life and human health.

In a formal Cooperative Agreement with EPA, the University of Wisconsin-Superior contracted to perform toxicity tests with selected "priority pollutants" utilizing various species of freshwater organisms in an effort to provide some of the data necessary for the development of water quality criteria statements for the protection of freshwater aquatic life. Toxicity tests were also conducted with several haloalkanes and halobenzenes closely related to "priority pollutants" and with methanol and dimethylformamide which are sometimes used as carrier solvents in toxicity tests.

Studies with mammalian systems have suggested that carbon tetrachloride, chloroform and other chlorinated alkanes are converted to toxic metabolites by the microsomal mixed function oxidase system of the liver (Docks and Krishna, 1976; Watanabe et al., 1978). However, information is limited concerning the metabolic disposition and protein binding of such compounds in fish and aquatic food chain organisms. Therefore, one aspect of this study was to investigate the comparative metabolism and protein binding potential of carbon tetrachloride, chloroform, 1,1,2-trichloroethylene, 1,1,2-trichloroethane and monochlorobenzene by microsomal fractions of rainbow trout (*Salmo gairdneri*) liver and the water flea (*Daphnia magna*).

SECTION II

CONCLUSIONS

Acute toxicity tests were conducted with fathead minnows (Pimephales promelas), rainbow trout (Salmo gairdneri), bluegill sunfish (Lepomis macrochirus), flagfish (Jordanella floridae), water fleas (Daphnia magna), scuds (Gammarus pseudolimnaeus), midge larvae (Tanytarsus dissimilis Johannsen 1937), and green algae (Selenastrum capricornutum). Fathead minnows were exposed to arsenic^{+3}, mercury^{+2}, silver^{+1}, dimethylformamide (DMF), and methanol with resulting estimated 96 hr LC_{50} values of 14.2, 0.150, 0.0107, 10,700, and 28,100 $mg \cdot L^{-1}$, respectively. Rainbow trout were exposed to hexachloroethane, tetrachloroethylene, tetrachloroethylene with DMF as a carrier solvent, DMF, 1,2-dichlorobenzene, 1,4-dichlorobenzene, 1,2,4-trichlorobenzene, pentachlorobenzene with DMF, hexachlorobenzene with DMF, hexachlorobutadiene, and methanol with resultant 96 hr LC_{50} estimates of 0.94, 4.99, 5.84, 10,000, 1.58, 1.12, 1.53, >0.71, >0.0809, 0.320, and 20,000 $mg \cdot L^{-1}$, respectively.

Bluegill sunfish were exposed to hexachlorobutadiene, hexachlorobenzene with DMF, DMF, and methanol with resultant 96 hr LC_{50} estimates of 0.324, >0.0784, 7,100, and 15,500 $mg \cdot L^{-1}$, respectively. Flagfish were exposed to arsenic^{+3} and silver^{+1} with resultant 96 hr LC_{50} estimates of 14.4 and 0.0092 $mg \cdot L^{-1}$, respectively.

Water fleas were exposed to hexachloroethane, pentachloroethane, 1,1,2,2-tetrachloroethane, 1,1,2-trichloroethane, 1,2-dichloroethane, 1,3-dichlorobenzene, 1,2,4-trichlorobenzene, tetrachloroethylene, di-n-butylphthalate, DMF, chlordane and nickel^{+2} with resultant unfed 48 hr LC_{50} estimates of 2.90, 7.32, 62.1, 186, 268, 7.43, 2.09, 18.1, 3.70, 14,530, 0.035, and 0.915 $mg \cdot L^{-1}$, respectively. LC_{50} estimates were also made for most of the same compounds in exposures where the organisms were fed. EC_{50} estimates were made for fed and unfed exposures with the chlorinated compounds and with arsenic^{+3}.

Scuds were exposed to pentachlorophenol, arsenic^{+3}, silver^{+1}, lead^{+2}, and chromium^{+6} with resultant 96 hr LC_{50} estimates of 280, 875, 4.49, 140, 67.1 and 94.1 $\mu g \cdot L^{-1}$, respectively. Midge larvae were exposed to hexachloroethane, tetrachloroethylene, 1,2-dichlorobenzene, 1,4-dichlorobenzene, hexachlorobenzene with DMF, pentachlorophenol, DMF, chromium^{+6}, lead^{+2}, silver^{+1}, selenium^{+4}, and cyanide with resultant 48 hr LC_{50} estimates of 5.85, 30.8, 12.0, 13.0, >0.0581, 46.0, 36,000, 57.3, 224, 3.17, 42.5, and 2.36 as HCN or 2.49 as CN^- $mg \cdot L^{-1}$, respectively.

Green algae were exposed to toxaphene and heptachlor for 96 hr with resultant EC_{50} estimates (50% reduction of growth) of 0.38 $mg \cdot L^{-1}$ for toxaphene and 38.1 and 28.2 $\mu g \cdot L^{-1}$ for two tests with heptachlor.

Chronic and subchronic toxicity tests were conducted using water fleas, fathead minnows, and flagfish. Water fleas were exposed to 1,1,2,2-tetrachloroethane, 1,1,2-trichloroethane, 1,2-dichloroethane, 1,2,4-trichlorobenzene, 1,3-dichlorobenzene, 1,2,4-trichlorobenzene, 1,3-dichlorobenzene, tetrachloroethylene, and arsenic^{+3} for 28 days with significant ($p \leq 0.05$ or $p \leq 0.01$) reductions in production of young at concentrations at or above 14.4, 41.8, 20.7, 0.694, 1.45, 1.11, and 1.32 $mg \cdot L^{-1}$, respectively.

Fathead minnows were exposed to arsenic^{+3} for 30 days post-fertilization and mercury^{+2} for 35 days post-fertilization. The "no-effect" concentration for arsenic^{+3} was between 2.1 and 4.3 $mg \cdot L^{-1}$ based upon significant ($p \leq 0.01$) reductions in wet weight and body length. Mercury^{+2} exposures resulted in significant ($p \leq 0.01$) reductions in wet weight and length at all exposure concentrations. A "no-effect" concentration for mercury^{+2} was less than the lowest exposure concentration of 0.23 $\mu g \cdot L^{-1}$. Flagfish were exposed to arsenic^{+3} for 30 days post-fertilization with a resultant "no-effect" concentration between 2.13 and 4.12 $mg \cdot L^{-1}$ based upon a reduction in body length.

Uptake, elimination, and metabolism of di-*n*-butylphthalate was studied with fathead minnows. A steady-state level of ^{14}C equivalents of di-*n*-butylphthalate was attained within 4 hr in the whole-body. Bioconcentration factors in ^{14}C equivalents of di-*n*-butylphthalate were 2,068 and 2,125 for the two measured exposure concentrations. Estimated bioconcentration factors for parent di-*n*-butylphthalate were 570 and 590 for the two tests based upon a mean value of 27.6% unmetabolized compound over an 11 day exposure.

Seven metabolites of di-*n*-butylphthalate were separated by thin-layer chromatography after three days of exposure. The only metabolite identified was phthalic acid.

Binding of di-*n*-butylphthalate to proteins was studied using rainbow trout liver microsomes and water flea post-mitochrondrial supernatant (PMS). Irreversible binding to proteins occurred with 9% of the compound bound to the rainbow trout liver microsomes in 2hr and <1% irreversibly bound to water flea PMS in 1 hr.

Microsomal metabolism and binding of carbon tetrachloride, chloroform, chlorobenzene, 1,1,2-trichloroethylene, and 1,1,2-trichloroethane were studied with rainbow trout liver microsomes and water flea PMS. The compounds were metabolized by both species with rainbow trout appearing to have a greater capacity for metabolizing them. The compounds were metabolized by rainbow trout in the following order: chloroform > 1,1,2-trichloroethane, > 1,1,2-trichloroethylene > chlorobenzene > carbon tetrachloride. The compounds were metabolized by water fleas in the following order: chloroform > chlorobenzene > 1,1,2-trichloroethylene > 1,1,2-trichloroethane $\approx$ carbon tetrachloride.

Mixed function oxidase assays were performed on the microsomal fraction from rainbow trout liver and the water flea PMS fraction. Rainbow trout liver microsomes had 0.28 and 0.19 $nM \cdot mg^{-1}$ of cytochrome P-450 and cytochrome b_5, respectively. The level of NADPH cytochrome c reductase activity was 16 nM of cytochrome c reduced$\cdot min^{-1} \cdot mg^{-1}$ protein. Water flea PMS had 42 nM of cytochrome c reductase activity$\cdot min^{-1} \cdot mg^{-1}$ protein.

SECTION III

RECOMMENDATIONS

Adequate assessment of a particular chemical's toxicity would be enhanced by exposing the chemical to many species of aquatic organisms representing the taxa likely to be impacted in the environment. Particular effort should be expended with species shown to be generally sensitive to the class of compound of immediate interest. Organisms such as *Tanytarsus dissimilis* (midge) with consistently high tolerances to a broad range of chemical classes should receive minimal effort.

Physical properties (i.e. hardness, pH, temperature) of the laboratory test water need to be carefully monitored, and several waters with different natural chemical characteristics used for exposures to assess the impacts of these parameters. The effects that water hardness and organic ligands have upon toxicity of some metals are known but not completely understood. Studies should be conducted to elucidate the relationships between chemical characteristics of natural waters and pollutant toxicity.

A better understanding is needed of compound metabolism and enzyme induction in aquatic organisms. Additional research on the capabilities of animals from various taxonomic groups to metabolize foreign chemicals would be valuable.

SECTION IV

METHODS

Water Supply and Environmental Control

Water for the toxicity tests was either directly from Lake Superior or was dechlorinated city water from Superior, WI. (Superior, WI derives its water from shallow wells beneath Lake Superior.) Several chemical parameters (dissolved oxygen, pH, hardness, acidity, and alkalinity) were monitored during the fish and scud toxicity tests by standard analytical methods (American Public Health Association, 1975). A portion of the water was heated before being distributed to the test systems. Lighting for the toxicity tests was artificial, supplied by fluorescent bulbs centered above the exposure chambers.

Test Organisms

Fathead minnow (*Pimephales promelas*) brood fish were received from stock maintained by the Environmental Research Laboratory-Duluth, MN, U.S. EPA. Brood fish were maintained at 25 C, and were fed twice daily a diet of frozen adult brine shrimp.

Asbestos pipe (12.5 cm O.D.) cut in half, longitudinally, was used as the spawning substrate. The spawning substrates (tiles) were checked daily for egg deposition. Eggs were removed from the tiles the same day ($\leq$ 24 hr) that spawning occurred when used in early life-stage tests. Eggs were allowed to remain on the tiles and were cared for by brood stock males until 50% or more were "eyed up" for later use in acute toxicity tests. Tiles with "eyed up" eggs were placed in temperature controlled (25 C) hatching chambers to complete incubation. Once they had hatched (approximately 96 hrs after spawning at 25 C), fry were transferred to rearing chambers where they were fed fresh, newly hatched brine shrimp nauplii (Artemia sp.) three times daily.

Rainbow trout (Salmo gairdneri) fingerlings used in the study were received from Lake Mills, WI National Fish Hatchery and from the Fattig Hatchery of Brady, NE. The fish were acclimated and maintained in Lake Superior water at 12 C until used in testing. They were maintained on a diet of Glencoe Mills trout chow.

Bluegill sunfish (Lepomis macrochirus) were obtained from the Newtown, OH Laboratory of the U.S. EPA. The fish were acclimated and maintained in Lake Superior water at 25 C. They were maintained on a diet of Glencoe Mills trout chow until used in acute tests.

Flagfish (Jordanella floridae) were obtained through brood stock maintained at the Environmental Research Laboratory-Duluth, MN,U.S. EPA. Brood stock were fed frozen adult brine shrimp. Young flagfish were fed freshly hatched brine shrimp and were raised in continuously flowing water at 25 C.

Stainless steel mesh grids covered with yarn were used as spawning substrates for flagfish brood parents. Eggs were removed from the yarn the same day ($\leq$ 24 hr) that spawning occurred when used in early life-stage tests. If the hatched fry were to be used for acute tests, the eggs and fry were handled as described for the fathead minnow.

Water flea (Daphnia magna) brood stock was obtained from the Environmental Research Laboratory-Duluth, MN,U.S. EPA. The brood stock was maintained at a temperature of approximately 20 C on a diet consisting of a mixture of finely ground trout chow and baker's yeast.

Scuds (Gammarus pseudolimnaeus) were collected from the Eau Claire River in Douglas County, WI. They were acclimated and reared in 56 L glass chambers with continuously flowing Lake Superior water at 20 C. The organisms were fed leaves of various deciduous species of trees native to St. Louis County, MN, that had been soaked in lake water for at least one month. Reproduction of the scuds occurred in the rearing chambers. At the start of a toxicity test organisms were selected based upon size uniformity with no attempt to determine age.

A midge (*Tanytarsus dissimilis* Johannson 1937) culture was maintained from stock organisms received from the Environmental Research Laboratory-Duluth, MN, U.S. EPA. The colony was maintained at a water temperature of approximately 20 C on a diet of Cerophyll® and trout pellets as described by Anderson *et al*. (1980).

A stock culture of green algae (*Selenastrum capricornutum*) was received from the Environmental Research Laboratory-Corvallis, OR, U.S. EPA. It was maintained in an environmental chamber according to the procedure of Miller *et al*. (1978) with some modifications. Modifications that were employed included a doubling of the nutrient solution concentration to allow for greater algal biomass production and stock culture transfers every 4-5 days.

Acute Toxicity Tests

Fathead Minnows - Fathead minnows were used as test organisms in acute tests with arsenic^{+3}, mercury^{+2}, and silver^{+1}. The test with arsenic^{+3} was conducted with dechlorinated city water while the mercury^{+2} and silver^{+1} tests were conducted with Lake Superior water. These were flow-through tests using a proportional diluter system (Mount and Brungs, 1967), in which there were five toxicant concentrations plus a control all in duplicate. Individual exposure chambers measured either 26 x 17 x 15 cm, or 20 x 35 x 15 cm and contained 3.1 L and 6.3 L of water. The diluter system delivered 0.5 L of fresh water alone to the controls or 0.5 L of fresh water plus toxicant in the case of the exposure groups every 15-19 min. Water temperature was maintained at 25 C and a 16 hr light photoperiod was used. Ten to 20 fish of age 30-32 days were placed into each chamber. Fish standard lengths and weights for the individual tests were: arsenic^{+3} - 21.0 ± 2.5 mm, 0.139 ± 0.140 g (n=20); mercury^{+2} - 20.0 ± 1.8 mm, 0.098 ± 0.027 g (n=20) and silver^{+1} - 19.2 ± 3.0 mm, 0.079 ± 0.031 g (n=40). Fish were not fed during the acute tests. Observations were made at regular intervals through 96 hrs for mortalities and other gross behavioral effects. Death was defined as cessation of opercular movement in all acute tests with fish.

Several water quality parameters were monitored throughout the tests. These included temperature, dissolved oxygen, hardness, alkalinity, acidity, and pH. These values are presented in Appendix A, Table A-1.

Toxicant concentrations were measured daily in acute tests. All twelve chambers were analyzed at 0 and 96 hr, with alternating replicates analyzed at 24, 48 and 72 hr. Measured concentrations of toxicants are presented in Appendix B, Table B-1.

Rainbow Trout - Rainbow trout were used as test organisms in acute tests with the following priority pollutants: tetrachloroethylene, 1,2-dichlorobenzene, 1,4-dichlorobenzene, hexachlorobenzene, and hexachlorobutadiene. Acute tests with rainbow trout and hexachloroethane, 1,2,4-trichlorobenzene, and pentachlorobenzene were also conducted.

All rainbow trout tests with the exception of penta- and hexachlorobenzene were flow-through tests conducted in the same type of diluter system as described for fathead minnows. The diluter cycled every 10-16 min, providing from 6.8 to 10.6 volume additions per day. Water temperature was maintained at approximately 12 C. Ten fish were tested per chamber. Fish were of the following sizes for the individual tests: tetrachloroethylene - 6.1 ± 1.0 cm, 3.2 ± 1.5 g (n=19); tetrachloroethylene with dimethylformamide (DMF) carrier-solvent 7.3 ± 1.0 cm, 5.86 ± 2.45 g (n=19); 1,2-dichlorobenzene - 5.6 ± 0.8 cm, 2.69 ± 1.24 g (n=10); 1,4-dichlorobenzene - 5.3 ± 0.6 cm, 2.1 ± 1.0 g (n=20); hexachlorobutadiene - 5.6 ± 0.6 cm, 2.6 ± 0.9 g (n=19); hexachloroethane - 6.6 ± 1.0 cm, 4.3 ± 1.8 g (n=20); 1,2,4-trichlorobenzene - 4.7 ± 0.4 cm, 1.6 ± 0.4 g (n=20).

Water quality parameters were routinely measured (Appendix A, Table A-1). Toxicant concentrations were measured daily as described with fathead minnows (Appendix B, Table B-1).

Penta- and hexachlorobenzene acute tests were conducted in a different type of flow-through system due to their limited water solubilities. Pentachlorobenzene was tested with dimethylformamide (DMF) as a carrier solvent. Pentachlorobenzene/DMF stock stolutions of known concentrations were pumped into 5 test chambers with fluid metering pumps. The control chambers received DMF only. DMF concentrations were nominally equal and averaged 395 $mg \cdot L^{-1}$ between exposure chambers. Test chambers were 30 x 60 x 30 cm, and contained 27 L of water (depth of 15 cm). Pumps were set to deliver every time the system cycled and delivered 1 L of Lake Superior water to each chamber. The cycle time of the system averaged 16.5 min, providing 3.2 volume additions of water per day. The mean water temperature was 12.7 C.

The test was run with 10 fish [mean standard length, 6.9 ± 1.2 cm; mean weight, 5.2 ± 2.5 g (n=20)] per chamber. Replicates were separated in time by 11 days. Since there were insufficient deaths at 96 hr to calculate an LC_{50}

concentration, the exposures were continued through 144 hr.

Hexachlorobenzene was tested in the system as described for pentachlorobenzene, also with DMF as a carrier-solvent. Two toxicant concentrations plus a control in duplicate were used. The lower concentration was at or near the solubility limit and the higher concentration exceeded water solubility. DMF concentrations were nominally equal in all chambers, and averaged 932 $mg \cdot L^{-1}$. Test duration was 96 hr at a mean water temperature of 11.2 C. The cycle time of the system averaged 8.25 min, providing 6.5 volume additions of water per day. Ten fish per tank were tested. Mean fish length and weight values were 3.3 ± 0.3 cm and 0.5 ± 0.1 g (n=20), respectively.

Toxicant concentrations were measured daily (Appendix B, Table B-1). Water quality parameters for penta- and hexachlorobenzene tests are presented in Appendix A, Table A-1.

Bluegill Sunfish - Bluegill sunfish were used as test organisms in acute tests with hexachlorobutadiene and hexachlorobenzene. In the test with hexachlorobutadiene, a proportional diluter system was used. Chamber dimensions were 51 x 15 x 15.5 cm, with a 10 cm water depth for a volume of 7.7 L. The diluter cycle time was 9.1 min, providing 10.3 volume additions per day. Ten fish were exposed per chamber. Mean standard length of the fish was 3.9 ± 0.5 cm, and mean weight was 1.5 ± 0.6 g (n=20). Water temperature was maintained at 25.2 C.

Hexachlorobenzene was tested with bluegill sunfish in the system as described for hexachlorobenzene and rainbow trout, with two toxicant concentrations and a control in duplicate. DMF was used as a carrier-solvent, and averaged 884 $mg \cdot L^{-1}$ in all chambers. The delivery system cycled every 9.7 min, providing 5.5 volume additions of water daily. The test was conducted at a mean water temperature of 23.3 C. Ten fish were tested per chamber at a mean standard length of 2.9 ± 0.4 cm and a mean weight of 0.4 ± 0.1 g (n=10). Water quality parameters and toxicant concentrations are presented in Appendices A and B, Tables A-1 and B-1, respectively.

Flagfish. Flagfish were used in acute tests with arsenic^{+3} and silver^{+1}. Flagfish 34 days of age (standard length, 13 ± 2.0 mm; weight, 0.058 ± 0.027 g, n=20) were exposed to arsenic^{+3} in a proportional diluter system using dechlorinated city water. They were tested simultaneously with fathead minnows in glass exposure chambers (20.5 x 30 x 25 cm) divided with screen into two sections. Flagfish sections contained an average of 2.1 L of water. Twenty fish per chamber were tested. The diluter cycle time of 19 min provided 6.9 volume additions of water per day. The test was conducted at a mean water temperature of 25.8 C.

Flagfish 30 days of age (standard length, 12.7 ± 1.6 mm; weight, 0.044 ± 0.021 g, n=30) were exposed to silver^{+1} in a proportional diluter system using Lake Superior water. They were tested simultaneously with fathead minnows in glass chambers (15 x 30 x 25 cm) divided by screen into two sections. Fifteen fish per chamber were tested. The diluter cycle time of 16 min provided about 8 volume additions of water per day. Mean test water temperature was 24.7 C. Water quality parameters and toxicant concentrations were measured routinely throughout the tests (Appendices A and B, Tables A-1 and B-1, respectively).

Daphnia magna - Daphnia magna was used as the test species in static acute tests conducted with 1,2-dichloroethane, 1,1,2-trichloroethane, 1,1,2,2-tetrachloroethane, pentachloroethane, hexachloroethane, tetrachloroethylene, 1,3-dichlorobenzene, 1,2,4-trichlorobenzene, di-*n*-butylphthalate, chlordane, nickel^{+2}, and arsenic^{+3}. Acute tests with chlorinated ethanes, ethylene, and benzene were conducted using first instar ($\leq$ 24 hr old) daphnids. Adult daphnids were originally obtained from the laboratory stock reared at the Environmental Research Laboratory-Duluth, MN. Both stock and test animals were maintained in a constant temperature water bath (20 ± 1 C).

A combination of Gro-Lux and Duro-Test (Optima FS) fluorescent bulbs provided 32 ft-candles of light at the air-water interface, and were set for a 16L:8D photoperiod coupled with a 15 min transition period between light and dark phases. All culturing and testing was done with Lake Superior water which was filtered (5 μm) and aerated. All chemical stock solutions were prepared by saturating lake water with the test chemical on a stirring plate.

Acute tests were conducted according to the "Proposed Standard Practice of Conducting Basic Acute Tests with Fishes, Macroinvertebrates, and Amphibians - Draft No. 8". Test containers were 200 mL erlenmeyer flasks filled to 200 or 160 mL for tests in which the daphnids were unfed or fed, respectively. The flasks were tightly stoppered with foil wrapped neoprene stoppers. Food concentration was 20 $mg \cdot L^{-1}$. The measure of acute toxicity was the 48 hr median effective concentration (48 hr EC_{50}) based upon complete immobilization and the 48 hr median lethal concentration (48 hr LC_{50}) based upon death as determined by cessation of heart beat and gut movement. Both were determined using a 30X dissection scope.

Toxicant concentrations were measured during each test (Appendix B, Table B-1). Water quality parameters are presented in Appendix A, Table A-2.

The Daphnia acute test with di-n-butylphthalate was a renewed static test using Lake Superior water, in which toxicant solutions were renewed daily, and test organisms were transferred daily by wide-mouthed pipettes into the new solutions. Exposures were conducted in 200 mL erlenmeyer flasks containing

150 mL of solution. Five first instar (<24 hr old) daphnids per duplicate flask were tested. The test organisms were obtained from the Environmental Research Laboratory-Duluth, MN (U.S. EPA) stock culture. Culture and test organisms were maintained at 20 ± 2 C. A 16L:8D photoperiod was used with a light intensity of 45 ft-candles. Flasks were observed daily for mortalities. Water quality parameters and toxicant concentrations are presented in Appendices A and B, Tables A-2 and B-1, respectively.

The _Daphnia_ acute static test with chlordane was conducted with Lake Superior water (first instar, <24 hr old from the Environmental Research Laboratory-Duluth, MN stock cultures). Ten organisms were placed into solutions in 200 mL erlenmeyer flasks. Five concentrations and a control were tested, in duplicate. The flasks were kept in a 21 C water bath, and a 16L:8D photoperiod was used. Flasks were observed daily for mortalities. Water quality parameters and toxicant concentrations were measured (Appendices A and B, Tables A-2 and B-1, respectively).

The _Daphnia_ acute test with nickel^{+2} was conducted in Lake Superior water, using 10 organisms (first instar <24 hr old, from the Environmental Research Laboratory-Duluth, MN stock culture) per flask. Five concentrations plus a control in duplicate, were tested in a 20 C water bath. The flasks were 300 mL erlenmeyers containing approximately 250 mL of water. A 16L:8D photoperiod was used, with a light intensity of 60 ft-candles. Water quality parameters and toxicant concentrations are presented in Appendices A and B, Tables A-2 and B-1, respectively. Flasks were observed daily for mortalities.

The _Daphnia_ acute test with arsenic^{+3} was conducted in Lake Superior water using 9-11 organisms (first instar, 24 ± 12 hr old, from the University of Wisconsin-Superior, WI stock culture) per flask. Six concentrations plus a control in duplicate were tested. The flasks were maintained at a temperature of 14.8 ± 0.8 C, with a photoperiod of 16L:8D. Acute tests were run with organisms both fed throughout exposure and not fed. Flasks were observed daily for mortalities. Water quality parameters and toxicant concentrations were measured (Appendices A and B, Tables A-2 and B-1, respectively).

Scuds - *Gammarus pseudolimnaeus* was tested in acute tests conducted with pentachlorophenol, arsenic^{+3}, silver^{+1}, lead^{+2}, and chromium^{+6}. All were flow-through tests conducted in proportional diluter systems (Mount and Brungs, 1967) using Lake Superior water.

In the pentachlorophenol test, 15 organisms ($\bar{x}$ = 0.050 ± 0.016 g) per chamber were tested. Chamber dimensions were 25.5 x 17 x 15 cm, with a water depth of 9 cm, for a volume of 3.9 L. The diluter cycled every 16 min, providing 11.5 volume additions per day. A 16L:8D photoperiod was used, with a light intensity of 20-31 ft-candles. The test was conducted at a water temperature of 17.1 ± 0.5 C. Five exposures plus a control, in duplicate, were used. Organisms were considered dead when movement ceased and they would not respond to prodding. Water quality parameters and toxicant concentrations were monitored throughout the test (Appendices A and B, Tables A-1 and B-1, respectively).

In the test with arsenic^{+3}, 10 organisms (0.3 - 1.1 cm, total length) per chamber were tested. Chamber dimensions were 6.3 x 6.3 x 9.3 cm. These chambers were placed inside larger glass chambers (26 x 17 x 15 cm) containing exposure water 8 cm deep. The inner chambers were constructed of glass on two sides and the bottom. Two sides were made of 202 Nitex® mesh to allow exchange of exposure water from the larger chambers. Exposure water passively entered each chamber containing the test organisms, and toxicant concentrations were identical both inside and outside of the inner chambers. The diluter cycle time averaged

16 min, providing 12.9 volume additions per day. The photoperiod and light intensity were the same as above. Water temperature was 18.4 ± 0.9 C. Five exposures, plus a control, in duplicate were tested. Water quality parameters and toxicant concentrations are presented in Appendices A and B, Tables A-1 and B-1, respectively.

An acute test with Gammarus and silver^{+1} was conducted under the same conditions as described for the arsenic^{+3} test. Ten organisms (0.3 - 1.1 cm, total length) per chamber were tested. The diluter cycle time was 15.8 min, providing 13.1 volume additions of water per day. The test water temperature was 19.9 ± 0.5 C. Water quality parameters and toxicant concentrations were monitored throughout the test (Appendices A and B, Tables A-1 and B-1, respectively).

An acute test with Gammarus and lead^{+2} was conducted under identical conditions as described for the arsenic^{+3} test. Fifteen organisms (0.053 ± 0.021 g) per chamber were tested at a water temperature of 17.6 ± 0.4 C. Water quality parameters and toxicant concentrations are presented in Appendices A and B, Tables A-1 and B-1, respectively.

Midges - Tanytarsus dissimilis was the test species for acute tests conducted with tetrachloroethylene, 1,2-dichlorobenzene, 1,4-dichlorobenzene, hexachlorobenzene, pentachlorophenol, hexachloroethane, chromium^{+6}, lead^{+2}, silver^{+1}, selenium^{+4}, and cyanide. All tests were conducted using Lake Superior water. Midge exposures were conducted in 8.5 cm diameter glass crystallizing dishes filled to a depth of 2.6 cm (∿200 mL volume) with test solutions. Each chamber with a 3 mm glass overflow tube contained lake water plus a small amount of food (ratio of 1L:0.0025 L), along with a fine layer of sand on the bottom. Food was a mixture of Cerophyll® and trout pellets blended with water.

Ten to 20 midge larvae in their 3rd or 4th instar stage of development (2.0 - 3.5 mm total length) were placed into each dish containing Lake Superior water, plus food, and were allowed to acclimate overnight (or for 48 hr in the silver^{+1} and selenium^{+4} tests) in a 20 C water bath. Hexachlorobenzene and pentachlorophenol tests were acclimated and run at room temperature (22.5 - 25.6 C). A photoperiod of 16L:8D at an intensity of 1.9 ft-candles was maintained throughout the test. In the case of the silver^{+1} test, the light was turned off at 24 hr due to an observable color change (graying) at the higher toxicant concentrations. In the selenium^{+4} test, the midges were exposed to room light only during working hours.

Each dish was examined after 24-48 hrs of acclimation for normal movement and case building. Midges were replaced if they were immobile or were building pupation cases. The water was siphoned off to a depth of 2-3 mm, and toxicant slowly dripped in from a separatory funnel at a rate of approximately 2 $mL \cdot min^{-1}$. Five toxicant concentrations plus a control, in duplicate, were tested, with the exception of hexachlorobenzene.

Hexachlorobenzene was tested at two concentrations plus a control, in duplicate. The crystallizing dishes contained 150 mL of solution and were covered. Nominal toxicant concentrations were 5.2 and 94.1 $\mu g \cdot L^{-1}$. DMF was used as a solvent carrier at a mean concentration of 1086 $mg \cdot L^{-1}$. The crystallizing dishes were twice siphoned and replaced with fresh hexachlorobenzene/DMF solutions at the beginning of the test in an attempt to maintain nominal concentrations. This test was run at a temperature of 23.9 ± 1.2 C.

Midges were observed on a light table at various time intervals through 48 hr. Effects and deaths were recorded. Death was defined as complete lack of movement when prodded.

The water was analyzed daily for toxicant concentrations (Appendix B, Table B-1). Water quality parameters were also monitored (Appendix A, Table A-1).

Green Algae - *Selenastrum capricornutum* was tested in 96 hr toxicity tests using toxaphene and heptachlor. Algal tests were conducted in 125 mL erlenmeyer flasks stoppered with foam plugs on a shaker platform. The flasks were placed in an environmental chamber and incubated under controlled conditions of light and temperature. Light intensity was 400 ft-candles and the test temperature was 24 C.

The nutrient solution concentration was twice the concentration used in the 1978 *Selenastrum* Bottle Test Procedure (Miller et al., 1978), providing for greater biomass production and more reliable dry weight measurements. Ethanol was used as a carrier-solvent in the toxaphene test, and all test flasks contained a concentration of 0.4% ethanol. No carrier-solvent was used in the heptachlor test.

Nutrient solutions containing toxaphene or heptachlor were inoculated with a 4-5 day-old culture of *Selenastrum* to yield an initial density of 20,000 $\text{cells}\cdot\text{mL}^{-1}$ in each flask. Triplicate flasks were inoculated at each exposure level (control plus 5 toxicant concentrations) for biomass determinations after 96 hr of exposure. After 96 hr, individual control and exposure flask solutions containing algae were filtered through pre-weighed 0.45 μm filters, dried, and weighed to determine algal biomass. Mean algal weights at various exposure levels were compared to the mean algal weight of the control group, and expressed as percentage inhibition of growth. The initial toxicant concentration that inhibited growth by 50% (EC_{50}) was determined from interpolation by the trimmed Spearman-Karber method.

Toxicant concentrations were measured during each test (Appendix B, Table B-1). A portion of each initial test concentration was analyzed to determine the starting concentration. One additional flask, which was not inoculated with algae, was carried through the 96 hr period for each exposure concentration. These flasks were used to determine final concentrations. Six additional flasks (containing no algae) were used at a middle toxicant concentration in each test. Three of these flasks were analyzed after 24 hr and the other three after 96 hr. Aliquots from the three flasks containing algae at the middle toxicant concentration for each test were filtered through glass fiber filters at the end of the test period to remove the algae to determine the amount of toxicant remaining in solution.

Chronic and Subchronic Toxicity Tests

Daphnia magna - *Daphnia magna* was used in chronic toxicity tests with 1,2-dichloroethane, 1,1,2-trichloroethane, 1,1,2,2-tetrachloroethane, tetrachloroethylene, 1,3-dichlorobenzene, 1,2,4-trichlorobenzene, and arsenic^{+3}. Chronic tests were conducted according to the ASTM "Proposed Standard Practice for Conducting Renewal Life Cycle Toxicity Tests with the Daphnid, *Daphnia magna*"(Draft No. 4, 1978), with minor modifications to control the losses of volatile chemicals. Test containers were 200 mL erlenmeyer flasks filled to 150-160 mL, with the exception of tetrachlorethylene which was filled to 175 mL. The flasks were tightly stoppered with foil wrapped neoprene stoppers to reduce chemical losses. Tests were conducted at a temperature of approximately 20 C with a 16L:8D photoperiod.

Tests with 1,1,2,2-tetrachloroethane, 1,3-dichlorobenzene, 1,2,4-trichlorobenzene and arsenic^{+3} had 7 replicates at each of 6 test chemical concentrations, whereas tests with 1,2-dichloroethane, 1,1,2-trichloroethane, and tetrachloroethylene had 10 replicates at each of 6 concentrations. Each flask initially contained 1 daphnid (<24 hr old). The food concentration was 20 $mg \cdot L^{-1}$.

Young daphnids were filtered from each flask after the transfer of adults and washed onto a watch glass to be counted alive. If less than 20 animals were present they were counted manually. If more than 20 animals were present, they were counted with an Artek™ counter. Chronic toxicity was determined by reproductive success and length of animals surviving the 28 day test. Length was determined using a 30X dissection scope and measuring from the top of the head to the base of the spine with an ocular microscope.

Toxicant concentrations were measured both before and after new solutions were added, and the mean of the "before" and "after" measurements represented the concentration for a particular time interval. Water quality parameters and toxicant concentrations are presented in Appendices A and B, Tables A-2 and B-1, respectively.

Fathead Minnows - Early life-stage toxicity tests were conducted using the fathead minnow as test species for tests with arsenic^{+3} and mercury^{+2}. The arsenic^{+3} test was performed using dechlorinated city water and the mercury^{+2} test with Lake Superior water. Both tests were performed in proportional diluter systems with five toxicant concentrations plus a control, all in duplicate. Test chambers for the arsenic^{+3} test contained an average of 1.9L of water, and had an average of 7.4 volume additions per day. Test chambers for the mercury^{+2} test contained 3.0 L of water, and had an average of 12.6 volume additions per day. The arsenic^{+3} test was conducted at a mean water temperature of 23.0 ± 2.7 C, and the mercury^{+2} test at a temperature of 25.0 ± 0.6 C.

Fathead minnow eggs $\leq$24 hr old were placed into incubation jars containing 200 µm mesh nylon screen bottoms. Fifty eggs per jar were added to each of 2 jars per treatment replicate. The eggs were incubated in the test chambers by slowly oscillating (6 revolutions per minute) the jars in the test solutions to enhance water movement past the eggs. Dead and fungused eggs were removed daily. Upon completion of hatch (5-6 days), dead, live and abnormal fry were counted, and healthy fry (20 total for the arsenic^{+3} test and 30 total for the mercury^{+2} test) were released into the test chambers. Feeding was begun the day after hatching and continued to the end of the test. Finely granualted dry fish food (Tetramin®) and newly hatched brine shrimp were fed for the entire test. Equal volumes of food were provided to each chamber.

Throughout the tests, observations were regularly made for mortalities and abnormal development. At the end of the test, all survivors were measured for wet weight and standard length to determine effects upon growth. Toxicant concentrations were measured in all tanks twice weekly (Appendix B, Table B-1). Water quality parameters were routinely measured throughout the tests (Appendix A, Table A-1).

Flagfish - Two early life-stage toxicity tests were conducted with arsenic^{+3} and flagfish. One was conducted using Lake Superior water and one using dechlorinated city water. Both tests were conducted in proportional diluter systems. The test with Lake Superior water was conducted in chambers containing 2.7 L of water, and had a mean of 16.7 volume additions of water daily. The test with dechlorinated city water was conducted in test chambers containing an average of 2.1 L with 7.4 volume additions daily. Mean water temperatures were 24.8 ± 1.3 C and 24.4 ± 2.8 C for tests in Lake Superior water and dechlorinated city water, respectively.

Flagfish eggs ($\leq$24 hr old) were placed into incubation jars as described for fathead minnows. In the test with Lake Superior water, 50 eggs per jar were added, while in the test with dechlorinated city water, 34 eggs per jar were added. In the test using city water, the eggs were treated for 10 min with a 0.4 $mg \cdot L^{-1}$ solution of malachite green on the first and second days of incubation to reduce fungal growth.

Upon hatching (6-7 days), the numbers of dead, live, and abnormal fry were determined, and 20 healthy fry were released into each chamber. Fish were fed Tetramin® once daily and newly hatched live brine shrimp 3 times daily. Equal volumes of food were provided to each chamber.

Throughout the test, observations were regularly made for mortalities and abnormal development. Weight (wet) measurements were taken at the conclusion of the test. Toxicant concentrations were measured in all tanks twice weekly (Appendix B, Table B-1). Water quality parameters are presented in Appendix A, Table A-1.

Toxicity Test Chemicals

The sources of test chemicals and chemical purity levels are presented in Appendix C, Table C-1. All chemicals had purity levels $\geq$95% except for chlordane and toxaphene, which were of technical grade.

Chemical Analysis of Toxicants

Organic Chemicals.

Exposure chamber concentrations of most of the organic chemicals tested were determined by extraction from water with an organic solvent (hexane, isooctane, or petroleum ether) and analysis by gas-liquid chromatography (GLC). GLC parameters used are presented in Appendix C, Table C-2. The extraction procedures and percentage recoveries are given in

Appendix C, Table C-3. Electron capture (^{63}Ni) detectors were used for most analyses on the different instruments.

Methanol concentrations in the exposure water for the rainbow trout and bluegill sunfish acute tests and dimethylformamide concentrations in the rainbow trout acute test were determined by GLC using direct aqueous injection on a Tenax GC® packed column and flame-ionization detection. Methanol concentrations in exposure chambers for the fathead minnow acute test were determined by using Rhodamine B dye as a tracer. The stock solution contained 25 $mg \cdot L^{-1}$ Rhodamine B which was delivered to the exposure tanks. The concentration of alcohol in the exposure tanks was calculated from the analysis of Rhodamine B.

Rhodamine B was measured using a Baird-Atomic Model SFR100 spectrofluorometer. The excitation and emission wavelengths were 554 nm and 578 nm, respectively. Standards were prepared in Lake Superior water and lake water was used as the reference blank. The exposure tanks contained from 0.1 to 1.3 $\mu g \cdot L^{-1}$ Rhodamine B.

Dimethylformamide concentrations in exposure chambers for acute tests with bluegill sunfish, fathead minnows, midges, and _Daphnia magna_ were determined by direct measurement on a spectrophotometer at a wavelength of 200 nm.

Inorganic Chemicals. Exposure chamber concentrations of arsenic, chromium, lead, mercury, nickel, silver and selenium were determined by atomic absorption analytical techniques. Samples were collected, preserved, and analyzed as stated in "Methods for Chemical Analysis of Water and Wastes" (U.S. EPA, 1979) and in "Analytical Methods for Atomic Absorption Spectrophotometry" (Perkin-Elmer Corp., 1976).

Arsenic, chromium, lead, nickel, selenium, and silver were analyzed with a Perkin-Elmer Model 306 atomic absorption spectrophotometer, employing a deuterium arc background corrector, an HGA-2100 graphite furnace atomizer or flame atomization burner head and a Model 56 recorder. Hollow cathode lamps were used for chromium, lead, nickel and silver. Electrodeless discharge lamps were used for arsenic and selenium. Flame or furnace atomization was used depending on sample concentration.

Mercury was analyzed on a Perkin-Elmer Model 403 atomic absorption spectrophotometer by a cold vapor technique (U.S. EPA, 1979), using a hollow cathode lamp. Methods of analyses and quality control parameters for the previous tests are summarized in Appendix C, Table C-4.

Cyanide was analyzed in the midge toxicity test exposure chambers by the colorimetric method described in "Standard Methods for the Examination of Water and Wastewater" (American Public Health Association, 1975). Absorbance readings were taken on a spectrophotometer at 578 nm.

Statistical Analysis of Test Results

LC_{50} values for all of the acute exposures with fish, scuds, and midges, and for some of the acute exposures with *Daphnia* were determined by the trimmed Spearman-Karber method (Hamilton *et al*., 1977). EC_{50} values for the algal toxicity tests with heptachlor and toxaphene were also determined by this method, with the EC_{50} values being the interpolated concentrations at which algal biomass was 50% of the biomass of controls. LC_{50} and EC_{50} values for the remainder of the *Daphnia* acute exposures were calculated by probit, moving average or binomial formulas depending upon the characteristics of the data.

Fish early life-stage test results and *Daphnia* chronic test results were analyzed by one-way analysis of variance and Dunnett's procedure (Steel and Torrie, 1960). "No effect" concentration ranges were determined for the parameters measured. The ranges encompass the highest mean exposure concentration at which no parameter was significantly affected ($p>0.05$) and the lowest mean exposure concentration at which one or more of the parameters was significantly affected ($p<0.05$).

Di-n-butylphthalate Uptake, Elimination and Metabolism

Fathead minnows were exposed in glass test chambers with inside dimensions of 29.5 x 59.0 x 29.5 cm, and a water depth of 15.0 cm. Each chamber contained 26.1 L of water. The chambers were glass covered and the outer sides were covered with fiber board to minimize visual disturbance to the fish.

The water delivery system was the water metering cell portion (w cells) of a proportional diluter (Mount & Brungs, 1967) adjusted to deliver 1 L of dilution water per cycle to mixing chambers before entering the exposure chambers. Each tank had a mixing chamber which was a 1 L beaker with a self-starting siphon which began to empty when nearly 1 L of dilution water entered. Simultaneously with the delivery of the dilution water to the mixing chamber, di-n-butylphthalate dissolved in methyl alcohol was delivered to the mixing chambers by metering pumps (Fluid Metering, Inc., Oyster Bay, NY, Model RPC, 0-1.6 $\text{mL}\cdot\text{min}^{-1}$). Methyl alcohol alone was delivered to the mixing chambers in the control tanks. The compound was mixed and then administered through a self-starting siphon to exposure chambers just beneath the water surface in a strong downward jet. This caused a mixing action within the test chambers. Concentrations of di-n-butylphthalate averaged 34.8 and 4.83 $\mu\text{g}\cdot\text{L}^{-1}$ for the higher and lower exposure concentrations, respectively. The delivery cycle time was 17 min.

Test water temperature was 25.4 ± 0.5 C (n=34). Hardness, alkalinity and acidity had mean concentrations of 44.3 ± 0.89 (n=2), 38.2 ± 0.35 (n=2), and 2.25 ± 0.071 (n=2) $\text{mg}\cdot\text{L}^{-1}$ as $CaCO_3$, respectively. Percent saturation of dissolved oxygen (Winkler Method) averaged 54.2 (n=2). The test water pH averaged 7.22 (n=2).

^{14}C labeled di-_n_-butylphthalate was supplied by California Bionuclear Corporation, 7654 San Fernando Road, Sun Valley, CA 91352, and had radiochemical purity of 98%. The compound was uniformly ring-labeled. Unlabeled phthalate was supplied by Monsanto and was 99.76% pure. Labeled and unlabeled stock compounds were mixed proportionally to yield a minimal activity of approximately 5000 counts$\cdot$min^{-1} in 100 mL of test water.

Di-_n_-butylphthalate (labeled and unlabeled) was dissolved in reagent grade methyl alcohol to give nominal low and high toxicant test concentrations of 5 and 50 $\mu g \cdot L^{-1}$, respectively. Approximately 0.1 mL (± 5%) of stock solution containing the test compound was mixed with 1.0 L of water before entering each chamber containing fish. The control chamber received 0.1 mL (± 5%) of methyl alcohol mixed with 1.0 L of water. This gave an approximate concentration of 100 $mg \cdot L^{-1}$ of methyl alcohol to all chambers, and 5 or 50 $\mu g \cdot L^{-1}$ of test compound to the low and high chambers, respectively.

Fathead minnows used for the study were from the EPA Environmental Research Laboratory-Duluth, MN stock culture. The fish used were 28-29 days old with an average weight of 0.091 ± 0.035 g (n=49). Fish were fed freshly hatched brine shrimp (_Artemia_ sp.) in equal volumes per tank during the study. Artificial lighting was supplied by florescent lamps with a 16L:8D photoperiod.

Before the exposure, fish were randomly withdrawn from a common pool of fish and placed into each test and control chamber. The chambers contained 130, 200, and 300 fish in the control, low, and high concentrations, respectively. Fish were exposed for 11 days, during which 80 fish from the high exposure concentration were saved for metabolite characterization, and the remaining fish transferred to clean water for depuration studies. Depuration lasted 21 days. Fish were randomly sampled from control and test chambers on day 0 at 0, 4, 8, and 12 hrs and on days 1, 2, 3, 5, 8 and 11 during uptake; and at 0, 4, 8, and 12 hrs on day 0 and on days 1, 2, 3, 4, 7, 14 and 21 during depuration.

Water samples were collected in duplicate with a glass siphon from near the center of the test chambers and approximately mid-depth in the water column. Water sampling dates coincided with fish sampling dates. Samples of test water (50 mL) were added to 100 mL volumetric flasks containing 50 mL of hexane. The samples were stirred vigorously for 45 min on a magnetic stirring plate, and allowed to stand for 15 min. A 5 mL aliquot of the solvent layer was added to 15 mL of scintillation cocktail (Permafluor® III, Triton® X-100 and scintillized toluene, 10:33:57 v/v) and counted in a Packard Model 3375 liquid scintillation spectrometer for 5.0 min.

Extraction efficiencies were measured several times during the testing period. Water was spiked with known amounts of radiolabeled stock solution and extracted. Aliquots of 5.0 mL from the solvent extract were directly added to 15.0 mL of scintillation cocktail and counted for 5.0 min. The counts were then compared to counts from equal amounts of radiolabeled stock solution added directly into the scintillation cocktail which contained 5.0 mL of hexane. Recovery from water was 97.6 ± 1.3% (n=19).

Fish were blotted dry on paper toweling and weighed to the nearest 0.1 mg. Whole fish were analyzed for ^{14}C content by oxidation in a Packard Sample Oxidizer (Model B306). Labeled carbon dioxide was trapped in Carbosorb® II and diluted with scintillation fluid. Recovery efficiencies were determined by adding radioactive stock compound to a combustion cup containing an unexposed fish. The recovery averaged 83.6 ± 10.1% (n=34).

Water and fish were counted for 5 min along with known concentrations of spiked samples. Concentration-count relationships were determined for each compound using 5 duplicated standards for both water and fish. Background counts and instrument quench curve readings were made with each run. A computer program corrected sample counts for differences in instrument quench readings, and calculated μg quantities of ^{14}C parent compound equivalents in water and fish based on a regression line fit (least squares) from parent compound standard curves. Sample concentrations were expressed in $\mu g \cdot L^{-1}$ of water or $\mu g \cdot g^{-1}$ of fish tissue.

Fish measured with unusually high and low concentrations of test compound (differing from the mean by more than one standard deviation) were statistically analyzed for outlying results ($p \leq 0.05$) by the method of Grubbs and Beck (1972). This resulted in the elimination of data on 4 fish out of the 285 analyzed.

Fish sampled from days 1, 9, 16, and 21, and also fish from 2 days after the test was completed were analyzed for lipid content. These samples were frozen until analysis. The fish were weighed and collectively homogenized with Na_2SO_4. The homogenate was extracted for 5 hr in a Soxhlet apparatus with a 1:1 mixture of methylene chloride and hexane. The extract was concentrated to 20 mL, and an aliquot placed in a rinsed, dried and pre-weighed weighing pan. The solvent was evaporated at 100 C, the lipid residue weighed, and percent lipids calculated.

Analytical Methods

Metabolism - Fathead minnows were sampled on days 0, 1, 2, 3, and 11 of uptake from the higher exposure concentration ($34.8\ \mu g \cdot L^{-1}$). A "pooled" weight of the fish from each day was taken and the fish were frozen until analysis.

Fish were thawed and homogenized in 5.0 mL of distilled water using a Potter-Elvehjem tissue homogenizer. The homogenate was centrifuged at 2,150 x g for 20 min. The supernatant was decanted and the pellet saved. A 50 μL aliquot of supernatant was analyzed for radioactivity using a Packard Tricarb Liquid Scintillation Counter (Model 3375). The remaining supernatant was frozen in liquid nitrogen and freeze-dried. The freeze-dried sample was dissolved in 0.1 mL of acetone, and chromatographed on glass thin-layer chromatography (TLC) plates. The TLC plates were coated with silica gel. Standards of di-n-butylphthalate and phthalic acid were spotted alongside the fish extract. The solvent system consisted of ethanol: H_2O: 25% acetic acid (100 mL:12 mL:16 mL). TLC plates were air dried and examined under short-wave ultraviolet light.

Radioactive spots and bands were also examined by autoradiography. The TLC plates were exposed to Kodak "No-Screen" X-ray plates for 6 weeks. The plates were processed for 5 min in Kodak X-ray developer, 10 min in Kodak fixer, and rinsed in distilled water for one-half hour. Radioactive areas appeared as dark bands or spots on the X-ray plates.

TLC plates from extracts of fish exposed for 0, 1, 3, and 11 days were used to quantify radioactivity for each day. Bands were numbered, then the silica gel was scraped off and added to scintillation cocktail. The samples were counted in a scintillation counter for 5 min.

Pellets remaining from the initial homogenates were analyzed for radioactivity by combustion with a Packard Sample Oxidizer. The samples were counted for 5 min by liquid scintillation technique.

Protein Binding - Radiolabeled (^{14}C) di-n-butylphthalate was incubated with a protein substrate and NADPH generating system to determine metabolism and irreversible protein binding of di-n-butylphthalate. Rainbow trout liver microsomes (4 mg protein, Lowry's Method) and Daphnia post-mitochondrial supernatant (PMS), (4 mg protein, Lowry's Method) were used as the protein substrates. The NADPH generating system consisted of 3 μM NADP, 30 μM glucose-6-phosphate, 1 unit of glucose-6-phosphate dehydrogenase, and 1 μM $MgCl_2$. The reaction mixture was brought to a total volume of 5.0 mL with 0.07 M sodium phosphate buffer, pH 7.45. The reaction mixture was incubated at 20 ± 2 C. Aliquots of reaction mixture were taken at 0, 15, 30, 45 and 60 min for Daphnia PMS, and 0, 15, 30, 45, 60 and 120 min for rainbow trout liver. Small portions of the reaction mixture were counted for total radioactivity. Reaction mixture aliquots were extracted twice with 15.0 mL of hexane. Protein in the reaction mixture was precipitated with 1.0 mL of 6M trichloroacetic acid, and the reaction mixture was then centrifuged at 2,150 x g for 20 min, forming a floating protein pellet. The floating protein pellet was added directly to scintillation cocktail. Aliquots of the hexane unextractable aqueous phase and hexane extract were added to scintillation cocktail. All samples were counted in a Packard Tricarb Liquid Scintillation Counter for 5 min. Data was analyzed with a Hewlett-Packard Automation Data System.

Microsomal Metabolism and Binding of Chlorinated Hydrocarbons by Rainbow Trout and Daphnia

Chemicals - Uniformly ^{14}C labeled 1,1,2-trichloroethylene and 1,1,2-trichloroethane were purchased from California Bionuclear Corporation, 7654 San Fernando Road, Sun Valley, CA 91352. Uniformly ^{14}C labeled 1-chlorobenzene, chloroform and carbon tetrachloride were purchased from New England Nuclear Corporation, 549 Albany Street, Boston, MA 02118. Purity of these compounds ranged between 98-99 percent as determined by gas-liquid chromatography. NADPH, NADP, glucose-6-phosphate monosodium salt, glucose-6-phosphate dehydrogenase from torula yeast, and cytochrome C were purchased from Sigma Chemical Company, St. Louis, MO.

Tissue Preparations - Livers were dissected from 3-5 rainbow trout (350-400 g), weighed, and cut into thin slices in cold (4 C) 0.15 M KCl solution. Liver slices were washed several times with KCl (0.15 M) to remove hemoglobin and red blood cells, transferred to 0.1 M pH 7.5 sodium phosphate buffer and homogenized by 6-8 passes of a teflon pestle in a Potter-Elvehjem glass homogenizer. Homogenates of 30-40% liver by weight in phosphate buffer were centrifuged twice at 10,000 x g for 15 min in a Beckman L5-50 ultra-centrifuge to remove nuclear and mitochondrial fractions, which were discarded. The 10,000 x g supernatant was centrifuged at 105,000 x g for 60 min using a T150 rotor. The supernatant was discarded and the pellet was stored at -20 C until used.

Adult *Daphnia* (approximately 21 days old) were reared in the laboratory form U.S. EPA Environmental Research Laboratory-Duluth, MN brood stock, and from 0.5 - 2.5 g (total wet weight) homogenized with a teflon pestle homogenizer. The homogenate was filtered through loose glass wool to remove chitinous materials, and centrifuged twice at 10,000 x g to remove nuclear and mitochondrial fractions. The PMS was then frozen at -20 C until used for *in vitro* metabolic studies.

Protein Determination - Protein determinations were made for *Daphnia* PMS and rainbow trout liver microsomes according to the method described by Lowry *et al*. (1951). This enabled known concentrations of protein to be used in the reaction mixture for metabolic studies.

In Vitro Metabolism Studies - Due to the highly volatile nature of carbon tetrachloride, chloroform, chlorobenzene, 1,1,2-trichloroethane and 1,1,2-trichloroethane, an incubation system was designed to study their binding to microsomal protein and their metabolism. This enclosed system consisted of an erlenmeyer flask (125 mL) which was fitted with a glass column (5 mm i.d.) containing two glass wool plugs with approximately 5 cm of silica gel between them to trap the parent compounds being volatilized from the reaction mixture. Another glass column connected the erlenmeyer flask to a CO_2 absorbing system containing a solution of Carbosorb II®. The reaction mixture in the erlenmeyer glask contained an NADPH-generating system (consisting of 3 μM glucose-6-phosphate, 1 unit[1] glucose-6-phosphate dehydrogenase, and 1 μM $MgCl_2$), 8 mg microsomal protein from rainbow trout liver or 4 mg PMS protein from Daphnia, in 0.07 M sodium phosphate buffer (pH 7.5) and 0.1 mL of test compound with known amount of radioactivity made to a final volume of 5 mL. The reaction mixture was incubated in a shaking water bath at a temperature of 24 ± 2 C. The reaction was initiated by addition of radioactive compound (0.1 mL) and was continued for 0, 15, 30, 45, 60 and 120 min with rainbow trout liver microsomes or 0, 15 and 30 min with PMS from Daphnia. The reaction was terminated at various time intervals by addition of 1 mL of 3 M trichloroacetic acid (TCA) solution. The reaction mixture was then extracted thrice with 10 mL of hexane and the extracts pooled. Total percent recovery was determined by summation of the radioactivity in the various fractions as compared to the known amount of radioactivity added initially. Recoveries ranged from 91.4 to 29.2% with recovery efficiency decreasing with time. The loss likely occurred by escape of ^{14}C through the silica gel column and the air space within the reaction vessel becoming saturated with parent compound or metabolites.

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[1] One unit will oxidize 1.0 μM of D-glucose 6-phosphate to 6-phospho-D-gluconate per min in the presence of NADP at pH 7.4 at 25 C.

Aliquots of the hexane extract (representing parent compound) were transferred to scintillation cocktail (10 mL Permaflour III®, 33 mL Triton X-100, 57 mL scintillized toluene) and ^{14}C radioactivity was counted with a Packard Model 3375 liquid scintillation spectrometer for 5 min. Background and quench corrections were made for all counts. The aqueous phase (representing water soluble metabolites) was then centrifuged at 2200 x g with an International Model PR-2 centrifuge for 20 min and the radioactivity determined in the supernatant and the floating protein pellet. This method distinguished between protein-bound and free radioactivity present in the aqueous phase which was unextractable in hexane.

The silica gel trap was extracted with 30 mL of hexane to determine the amount of radioactivity volatilized from the reaction mixture. The carbon dioxide absorbing solution was counted to determine radioactivity evolved as CO_2 during metabolic reactions or volatilized as parent compound. The analysis was performed three times with three batches of tissue preparations.

Enzyme Activity - Cytochrome P-450 and cytochrome b_5, were determined by difference spectroscopy with a Beckman DB-G spectrophotometer according to the methods of Omura and Sato (1964). NADPH-cytochrome c reductase activity was determined by the method described by Williams and Kamin (1962). Aniline hydroxylase activity was determined by measuring the amount of p-aminophenol produced during a 30 min incubation of the liver microsomes on the PMS with aniline hydrochloride at 24 C. The reaction mixture contained an NADPH-generating system as described previously, 1 μM of aniline hydrochloride and 5 mg microsomal protein. The reaction was stopped by addition of 0.5 mL of 3 M TCA. After centrifugation of the reaction mixture at 2200 x g for 20 min, a 1 mL aliquot of the reaction mixture was made basic with 0.5 mL of 10% Na_2CO_3

and a blue phenol-indophenol complex was formed by addition of 1 mL of 2% phenol in 0.2 N NaOH. Absorbance was measured using a Beckman DB-G spectrophotometer at 630 nm.

SECTION V

RESULTS

Acute Toxicity Tests

Fathead Minnows - 96 hr LC_{50} values with 95% confidence intervals for pooled replicate data in acute tests with arsenic^{+3}, mercury^{+2}, silver^{+1}, dimethylformamide, and methanol were 14.2 (12.5-16.0), 0.150 (0.128-0.176), 0.0107 (0.0106-0.0108), 10,700 (10,500-10,900), and 28,100 (27,200-29,000) $mg \cdot L^{-1}$, respectively (Table 1).

Rainbow Trout - LC_{50} values at selected time intervals for rainbow trout were determined for the following chemicals: hexachloroethane, tetrachloroethylene, tetrachloroethylene with dimethylformamide (DMF) as carrier solvent, DMF, 1,2-dichlorobenzene, 1,4-dichlorobenzene, 1,2,4-trichlorobenzene, hexachlorobutadiene, and methanol (Table 2). Values for 96 hr LC_{50}s ranged from 0.320 $mg \cdot L^{-1}$ for hexachlorobutadiene to 20,100 $mg \cdot L^{-1}$ for methanol. LC_{50} values at 96 hr were not calculable for pentachloro- and hexachlorobenzene due to insufficient mortalities at the highest exposure concentrations tested of 0.71 and 0.0809 $mg \cdot L^{-1}$, respectively.

Bluegill Sunfish - The pooled 96 hr LC_{50} with 95% confidence interval for bluegill sunfish exposed to hexachlorobutadiene was 0.324 (0.312-0.337) $mg \cdot L^{-1}$ (Table 3). Mortalities were insufficient to yield an LC_{50} value at 96 hr with hexachlorobenzyne/DMF. Only 1 of 20 fish died at the highest mean exposure concentration of 78.4 $\mu g \cdot L^{-1}$. Dimethylformamide and methanol produced 96 hr

TABLE 1. LC_{50} VALUES (95% CONFIDENCE INTERVALS) FOR POOLED REPLICATES OF ACUTE TESTS IN WHICH FATHEAD MINNOWS (*Pimephales promelas*) WERE EXPOSED TO ARSENIC^{+3}, MERCURY^{+2}, SILVER^{+1}, DIMETHYLFORMAMIDE AND METHANOL.

	LC_{50} (mg·L^{-1})			
Chemical	24 hr	48 hr	72 hr	96 hr
Arsenic^{+3}	19.0 (17.4-20.7)	15.9 (14.3-17.8)	14.7 (13.1-16.5)	14.2 (12.5-16.0)
Mercury^{+2}	0.240 (0.181-0.317)	0.196 (0.170-0.226)	0.155 (0.132-0.181)	0.150 (0.128-0.176)
Silver^{+1}	0.0152 (0.0137-0.0168)	0.0116 (0.0109-0.0124)	0.0107 (0.0107-0.0107)	0.0107 (0.0106-0.0108)
Dimethyl-formamide	11,500 (10,900-12,000)	10,800 (10,500-11,100)	10,700 (10,500-10,900)	10,700 (10,500-10,900)
Methanol	28,400 (27,600-29,200)	28,400 (27,600-29,200)	28,400 (27,600-29,200)	28,100 (27,200-29,000)

TABLE 2. LC_{50} VALUES (95% CONFIDENCE INTERVALS) FOR POOLED REPLICATES OF ACUTE TESTS IN WHICH RAINBOW TROUT (*Salmo gairdneri*) WERE EXPOSED TO SELECTED ORGANIC COMPOUNDS.

Chemical	LC_{50} ($mg \cdot L^{-1}$) 24 hr	48 hr	72 hr	96 Hr	192 hr
Hexachloroethane	1.80 (1.69-1.92)	1.17 (1.09-1.26)	1.05 (0.96-1.15)	0.94 (0.85-1.04)	0.77(0.72-0.83)
Tetrachloroethylene	4.99 (4.73-5.27)	4.99 (4.73-5.27)	4.99 (4.73-5.27)	4.99 (4.73-5.27)	n.d.[a]
Tetrachloroethylene/DMF	6.31 (5.54-7.18)	5.95 (5.23-6.78)	5.81 (5.06-6.67)	5.84 (5.05-6.76)	n.d.
Dimethylformamide (DMF)	11,000 (11,000-11,000)	10,000 (9,100-11,000)	10,000 (9,100-11,000)	10,000 (9,100-11,000)	n.d.
1,2-Dichlorobenzene	1.65 (1.49-1.84)[b]	1.58 (1.44-1.73)	1.58 (1.44-1.73)	1.58 (1.44-1.73)	1.54 (1.42-1.68)[c]
1,4-Dichlorobenzene	1.37 (1.25-1.49)	1.24 (1.13-1.35)	1.24 (1.13-1.35)	1.12 (1.05-1.20)	n.d.
1,2,4-Trichlorobenzene	2.30 (2.17-2.43)	2.00 (1.84-2.17)	1.73 (1.54-1.94)	1.53 (1.35-1.73)	1.28 (1.11-1.47)
Pentachlorobenzene/DMF	n.c.[d]	n.c.	n.c.	n.c.	0.28 (0.21-0.37)[c]
Hexachlorobenzene/DMF	n.c.	n.c.	n.c.	n.c.	n.d.
Hexachlorobutadiene	n.c.	n.c.	0.429 (0.372-0.495)	0.320 (0.268-0.381)	0.121 (0.098-0.149)
Methanol	20,300 (19,800-20,700)	20,100 (19,500-20,700)	20,100 (19,500-20,700)	20,100 (19,500-20,700)	n.d.

[a] Not determined.

[b] 22 hr LC_{50} value.

[c] 144 hr LC_{50} value.

[d] LC_{50} value not calculable due to insufficient mortalities at exposure concentrations tested.

TABLE 3. RESULTS FROM FLOW-THROUGH MEASURED ACUTE TOXICITY TESTS IN WHICH BLUEGILL SUNFISH (*Lepomis macrochirus*) WERE EXPOSED TO HEXACHLOROBUTADIENE, HEXACHLOROBENZENE/DMF, DIMETHYLFORMAMIDE, AND METHANOL (REPLICATES POOLED).

Chemical	LC_{50} (mg·L^{-1}) 24 hr	48 hr	72 hr	96 hr	192 hr
Hexachlorobutadiene	n.c.[a]	n.c.	0.387 (0.322-0.466)	0.324 (0.312-0.337)	0.318 (0.318-0.318)
Hexachlorobenzene/DMF	n.c.	n.c.	n.c.	n.c.	n.d.[b]
Dimethylformamide	7,500 (7,200-7,800)	7,500 (7,200-7,800)	7,400 (7,000-7,700)	7,100 (6,700-7,500)	n.d.
Methanol	19,230 (17,310-21,360)	19,230 (17,310-21,360)	17,720 (15,510-20,240)	15,500 (13,540-17,740)	n.d.

[a] LC_{50} value not calculable due to insufficient mortalities at exposure concentrations tested.

[b] Not determined.

LC_{50} values of 7,100 (6,700-7,500) $mg \cdot L^{-1}$ and 15,500 (13,540-17,740) $mg \cdot L^{-1}$, respectively.

Flagfish - Pooled 96 hr LC_{50} values (95% confidence interval) for acute tests with arsenic^{+3} and silver^{+1} were 14.4 (12.7-16.3) and 0.0092 (0.0080-0.0107) $mg \cdot L^{-1}$, respectively (Table 4).

Daphnia magna - LC_{50} values (48 hr, unfed) for Daphnia magna exposed to thirteen selected test chemicals ranged from 0.035 (0.032-0.038) $mg \cdot L^{-1}$ for chlordane to 14,530 (13,260-15,920) $mg \cdot L^{-1}$ for dimethylformamide (Table 5). EC_{50} values in addition to LC_{50} values were determined for eight chlorinated ethanes, benzenes and ethylene. EC_{50} values (48 hr, unfed) ranged from 2.10 (1.82-2.45) $mg \cdot L^{-1}$ for hexachloroethane to 155 (137-188) $mg \cdot L^{-1}$ for 1,2-dichloroethane.

Generally, little difference was noted between endpoints (LC_{50} or EC_{50}) whether test animals were fed or not fed during the exposures. Two exceptions were tetrachloroethylene, in which the LC_{50} value in the unfed test was twice that for the fed test, and arsenic^{+3}, in which the EC_{50} value for the fed test was about 3 times the value for the unfed test.

Gammarus pseudolimnaeus - LC_{50} values (96 hrs) for scuds exposed to pentachlorophenol, arsenic^{+3}, silver^{+1}, lead^{+2}, and chromium^{+6} ranged from 4.49 (3.67-5.49) $\mu g \cdot L^{-1}$ for silver to 875 (846-904) $\mu g \cdot L^{-1}$ for arsenic^{+3} (Table 6). The static chromium^{+6} test that was run with nominal exposure concentrations resulted in a 96 hr LC_{50} value (94.1 $\mu g \cdot L^{-1}$) that was approximately 40% higher than the value of 67.1 $\mu g \cdot L^{-1}$ for the chromium^{+6} test that was run in a flow-through system with measured exposure concentrations.

Tanytarsus dissimilis - LC_{50} values (48 hr) for midges exposed to hexachloroethane, tetrachloroethylene, 1,2-dichlorobenzene, 1,4-dichlorobenzene, TABLE 4. LC_{50} VALUES (95% CONFIDENCE INTERVALS) FOR POOLED REPLICATES OF ACUTE TESTS IN WHICH FLAGFISH (*Jordanella floridae*) WERE EXPOSED TO ARSENIC^{+3} AND SILVER^{+1}.

	LC_{50} ($mg \cdot L^{-1}$)			
Chemical	24 hr	48 hr	72 hr	96 hr
Arsenic^{+3}	18.3 (17.0-19.7)	16.3 (14.7-18.0)	15.9 (14.3-17.8)	14.4 (12.7-16.3)
Silver^{+1}	0.0441 (0.0418-0.0465)	0.0259 (0.0222-0.0301)	0.0138 (0.0119-0.0160)	0.0092 (0.0080-0.0107)

TABLE 5. 48 HR LC_{50} AND EC_{50} VALUES (95% CONFIDENCE INTERVALS) FOR POOLED REPLICATES OF DAPHNIA MAGNA EXPOSED TO SELECTED TEST CHEMICALS.

	LC 50		EC 50	
	Unfed	Fed	Unfed	Fed
Chemical	(mg·L^{-1})		(mg·L^{-1})	
Hexachloroethane	2.90[c] (2.50-3.33)	2.35[a] (1.99-2.86)	2.10[c] (1.82-2.45)	1.81[b] (1.61-2.07)
Pentachloroethane	7.32[c] (5.98-8.99)	8.02[c] (6.89-9.39)	4.69[c] (3.99-5.50)	6.88[c] (6.07-7.85)
1,1,2,2-Tetrachloroethane	62.1[b] (55.9-70.7)	56.9[c] (49.9-66.3)	23.0[a] (16.3-34.5)	25.2[c] (22.2-28.2)
1,1,2-Trichloroethane	186[c] (164-214)	174[c] (154-201)	80.6[a] (57.5-113)	77.8[a] (56.6-107)
1,2-Dichloroethane	268[b] (246-293)	315[c] (265-414)	155[a] (137-188)	183[b] (154-225)
1,3-Dichlorobenzene	7.43[c] (6.29-8.77)	7.23[c] (6.14-8.50)	4.23[a] (3.28-5.89)	5.98[a] (4.85-9.53)
1,2,4-Trichlorobenzene	2.09[c] (1.80-2.63)	1.68[b] (1.52-1.85)	n.d.[e]	n.d.
Tetrachloroethylene	18.1[b] (15.5-21.8)	9.09[b] (7.70-11.0)	8.50[a] (7.00-11.5)	7.49[b] (6.08-9.03)
Di-n-butylphthalate	3.70[d] (3.70-3.70)	n.d.	n.d.	n.d.
Dimethylformamide	14,530 (13,260-15,920)[d]	n.d.	n.d.	n.d.
Chlordane	0.035[d] (0.032-0.038)	n.d.	n.d.	n.d.
Nickel^{+2}	0.915[d] (0.782-1.070)	n.d.	n.d.	n.d.
Arsenic^{+3}	n.d.	n.d.	1.54[d] (1.20-1.97)	4.83[d] (3.71-6.29)

[a] LC_{50} or EC_{50} calculated by binomial method.

[b] LC_{50} or EC_{50} calculated by moving average method.

[c] LC_{50} or EC_{50} calculated by probit method.

[d] LC_{50} or EC_{50} calculated by trimmed Spearman-Karber method.

[e] No determinations were made.

TABLE 6. LC_{50} VALUES (95% CONFIDENCE INTERVALS) FOR POOLED REPLICATES OF ACUTE TESTS IN WHICH SCUDS (Gammarus pseudolimnaeus) WERE EXPOSED TO PENTACHLOROPHENOL, ARSENIC^{+3}, SILVER^{+1}, LEAD^{+2}, AND CHROMIUM^{+6}.

Chemical	LC_{50} ($\mu g \cdot L^{-1}$)			
	24 hr	48 hr	72 hr	96 hr
Pentachlorophenol	600 (540-650)[a]	400 (350-460)[b]	290 (250-340)	280 (240-330)
Arsenic^{+3}	n.c.[c]	1990 (1780-2220)[d]	875 (846-904)	875 (846-904)
Silver^{+1}	4.71 (3.82-5.79)[e]	4.49 (3.67-5.49)	4.49 (3.67-5.49)	4.49 (3.67-5.49)
Lead^{+2}	n.c.	275 (234-322)	n.d.[f]	140 (140-140)
Chromium^{+6}	n.c.	288 (252-329)[g]	164 (139-192)[h]	67.1 (55.0-81.8)
Chromium^{+6} static[i]	n.c.	809 (680-962)	415 (313-551)	94.1 (65.1-135.8)

[a] 26 hr LC_{50} value.

[b] 50 hr LC_{50} value.

[c] Not calculable due to insufficient mortalities at highest exposure.

[d] 43.5 hr LC_{50} value.

[e] 22.0 hr LC_{50} value.

[f] Not determined.

[g] 51.5 hr LC_{50} value.

[h] 73.5 hr LC_{50} value.

[i] Test run with nominal exposure concentrations.

hexachlorobenzene/DMF, pentachlorophenol, dimethylformamide, chromium^{+6}, lead^{+2}, silver^{+1}, selenium^{+4}, and cyanide ranged from 2.36 (2.10-2.66) $mg \cdot L^{-1}$ for cyanide to 36,000 (33,000-40,000) $mg \cdot L^{-1}$ for dimethylformamide (Table 7). Insufficient mortalities occurred with hexachlorobenzene to yield an LC_{50} value at 48 hr.

Selenastrum capricornutum - Exposure of Selenastrum to initial toxaphene concentrations of from 0.25 to 1.93 $mg \cdot L^{-1}$ resulted in growth inhibition ranging from 30.4 to 88.8% (Table 8). The 96 hr EC_{50} value for green algae (50% reduction in dry weight as compared to controls) exposed to toxaphene was 0.38 $mg \cdot L^{-1}$, based on initial toxaphene concentrations.

Exposure of Selenastrum to initial concentrations of heptachlor from 8.6 to 107 $\mu g \cdot L^{-1}$ reduced growth from 2.4 to 85.3% (Tables 9 and 10). The 96 hr EC_{50} values for algae exposed to heptachlor were 38.1 and 28.2 $\mu g \cdot L^{-1}$ based on initial heptachlor concentrations for tests 1 and 2, respectively. The heptachlor conversion product 1-hydroxychlordene was readily formed and had initial concentrations as high as 70.9% of the initial heptachlor concentrations. 1-Hydroxychlordene concentrations did not vary much over the 96 hr exposure. Heptachlor concentrations decreased to less than 0.8 $\mu g \cdot L^{-1}$ at all exposure levels.

Chronic and Subchronic Toxicity Tests

Daphnia Magna - Production of young Daphnia magna was significantly ($p \leq 0.05$ or $p \leq 0.01$) reduced by the test chemicals at or above the following mean concentrations (Table 11): 1,1,2,2-tetrachloroethane (14.4 $mg \cdot L^{-1}$), 1,1,2-trichloroethane (41.8 $mg \cdot L^{-1}$), 1,2-dichloroethane (20.7 $mg \cdot L^{-1}$), 1,2,4-trichlorobenzene (0.694 $mg \cdot L^{-1}$), 1,3-dichlorobenzene (1.45 $mg \cdot L^{-1}$), tetrachloro-

TABLE 7. 48 HR LC_{50} VALUES (95% CONFIDENCE INTERVALS) FOR POOLED REPLICATES OF ACUTE TESTS IN WHICH MIDGES (*Tanytarsus dissimilis*) WERE EXPOSED TO SELECTED INORGANIC AND ORGANIC CHEMICALS

Chemical	LC_{50} ($mg \cdot L^{-1}$) 24 hr	 48 hr	 72 hr
Hexachloroethane	n.c.[a]	5.85 (3.77-9.09)	1.68 (1.31-2.14)
Tetrachloroethylene	54.6 (47.4-62.8)	30.8 (28.7-33.0)	n.d.[b]
1,2-Dichlorobenzene	19.9 (16.7-23.7)	12.0 (10.0-14.5)	n.d.
1,4-Dichlorobenzene	22.1 (19.2-25.6)	13.0 (10.9-15.6)	n.d.
Hexachlorobenzene/DMF	n.c.	n.c.[c]	n.d.
Pentachlorophenol	84.8 (71.6-100)	46.0 (39.0-54.3)	n.d.
Dimethylformamide	47,000(43,000-51,000)	36,000(33,000-40,000)	n.d.
$Chromium^{+6}$	206 (167-254)	57.3 (46.4-70.8)	n.d.
$Lead^{+2}$	n.c.	224 (108-468)	n.d.
$Silver^{+1}$	5.03 (4.47-5.65)	3.16 (2.49-4.01)	n.d.
$Selenium^{+4}$	56.7 (56.7-56.7)	42.5 (36.7-49.2)	n.d.
Cyanide (free cyanide as HCN)	6.02 (5.75-6.30)	2.36 (2.10-2.66)	n.d.
Cyanide (free cyanide as CN^-)	8.99 (8.46-9.55)	2.49 (2.20-2.82)	n.d.

[a] Not calculable due to insufficient mortalities at highest exposure.

[b] Not determined.

[c] The highest concentration tested was 0.0581 $mg \cdot L^{-1}$.

TABLE 8. PERCENT INHIBITION OF *SELENASTRUM CAPRICORNUTUM* GROWTH WHEN EXPOSED TO SEVERAL CONCENTRATIONS OF TOXAPHENE FOR 96 HR.

Nominal Conc. ($mg \cdot L^{-1}$)	Initial Conc. ($mg \cdot L^{-1}$)	Final Conc. ($mg \cdot L^{-1}$)	Mean Conc. ($mg \cdot L^{-1}$)	Mean Algal Weight ± S.D. (g)	Mean % Inhibition
0.0	0.0	0.0	0.0	0.00240 ± 0.0005	0.0
0.3	0.25	0.14	0.195	0.00167 ± 0.0002	30.4
0.5	0.48	0.23	0.355	0.00085[a]	64.6
1.0	0.85	0.68	0.765	0.00057 ± 0.0002	76.3
1.5	0.87	0.64	0.755	0.00050 ± 0.0001	79.2
2.0	1.93	1.56	1.745	0.00027 ± 0.0002	88.8

a/ Only two samples were included for consideration at this concentration.

TABLE 9. PERCENT INHIBITION OF SELENASTRUM CAPRICORNUTUM GROWTH AT 96 HR FOLLOWING EXPOSURE TO SEVERAL CONCENTRATIONS OF HEPTACHLOR AND ITS BREAKDOWN PRODUCT, 1-HYDROXYCHLORDENE (Test 1)

Nominal Heptachlor Conc. (% of Stock Solution)	Heptachlor Conc. ($\mu g \cdot L^{-1}$)			1-Hydroxychlordene Conc. ($\mu g \cdot L^{-1}$)			Mean Algal Wt. ± S.D. (g)	Mean % Inhibition
	Initial	24 hr	Final	Initial	24 hr	Final		
0 (control)	<0.8		<0.8	<1.0		<1.0	0.0042 ± 0.0006[a]	0.0
20%	8.6		<0.8	6.1		6.1	0.0038 ± 0.0004[a]	9.5
40%	17.6		<0.8	11.5		14.9	0.0041 ± 0.0003	2.4
60%[b]	28.4	<0.8	<0.8	14.8	21.4	19.9	0.0040 ± 0.0007	4.8
80%	38.5		<0.8	17.8		20.1	0.0020 ± 0.0008	52.4
100%	44.4		<0.8	30.5		28.7	0.0012 ± 0.0006	71.4

a/ Only two samples were included for consideration at this concentration.

b/ Heptachlor and 1-hydroxychlordene concentrations are means of triplicate determinations at this exposure. Other exposure concentrations are from single determinations.

TABLE 10. PERCENT INHIBITION OF SELENASTRUM CAPRICORNUTUM GROWTH AT 96 HR FOLLOWING EXPOSURE TO SEVERAL CONCENTRATION OF HEPTACHLOR AND ITS BREAKDOWN PRODUCT, 1-HYDROXYCHLORDENE (Test 2)

Nominal Heptachlor Conc. (% of Stock Solution)	Heptachlor Conc. (µg·L^{-1})			1-Hydroxychlordene Conc. (µg·L^{-1})			Mean Algal Wt. ± S.D. (g)	Mean % Inhibition
	Initial	24 hr	Final	Initial	24 hr	Final		
0 (control)	<0.8		<0.8	<1.0		<1.0	0.0034 + 0.0010	0.0
20%	13.6		<0.8	4.5		6.7	0.0032 + 0.0007	5.9
40%	22.8		<0.8	11.4		13.2	0.0017 + 0.0004	50.0
60%[a/]	44.4	<0.8	<0.8	17.0	18.4	20.5	0.0013 + 0.0003	61.8
80%	57.0		<0.8	25.3		28.6	0.0009 + 0.0003	73.5
100%[b/]	107		<0.8	27.8		23.7	0.0005 + 0.0004	85.3

[a/] Heptachlor and 1-hydroxychlordene concentrations are means of triplicate determinations at this exposure. Other exposure concentrations are from single determinations.

[b/] This exposure level was not included in claculation of the EC_{50} value due to the unusually high analytical value for the initial concentration of heptachlor.

TABLE 11. MEAN EXPOSURE CONCENTRATIONS OF SELECTED TEST CHEMICALS AND EFFECTS UPON REPRODUCTIVE SUCCESS AND GROWTH IN *DAPHNIA MAGNA* DURING 28 DAY CHRONIC TESTS

Compound	Chemical Concentration $mg \cdot L^{-1}$ ($\bar{x}$ ± s.d.)	Number of Young Produced Per Adult ($\bar{x}$ ± s.d.)	Length (mm) of Adults ($\bar{x}$ ± s.d.)
1,1,2,2-Tetrachloroethane	0.0 (Controls)	162 ± 49	No data
	0.419 ± 0.036	84 ± 50	No data
	0.859 ± 0.085	69 ± 39	No data
	1.71 ± 0.17	71 ± 40	No data
	3.43 ± 0.39	78 ± 37	No data
	6.85 ± 0.90	78 ± 18	No data
	14.4 ± 1.4	23 ± 5**	No data
1,1,2-Trichloroethane	0.0 (Controls)	150 ± 42	4.1 ± 0.2
	1.72 ± 0.16	95 ± 53	3.9 ± 0.2
	3.40 ± 0.29	132 ± 57	3.8 ± 0.2
	6.35 ± 0.52	146 ± 55	4.1 ± 0.2
	13.2 ± 1.7	163 ± 59	4.0 ± 0.2
	26.0 ± 2.2	114 ± 31	3.9 ± 0.2*
	41.8 ± 3.0	11 ± 4**	3.9 ± 0.2*
1,2-Dichloroethane	0.0 (Controls)	164 ± 45	3.9 ± 0.3
	10.6 ± 0.8	128 ± 37	3.9 ± 0.2
	20.7 ± 1.7	88 ± 51*	3.8 ± 0.2
	41.6 ± 2.4	54 ± 24**	3.6 ± 0.2
	71.7 ± 4.8	43 ± 22**	3.4 ± 0.2**
	94.4 ± 5.5	19 ± 21**	3.1 ± 0.4**
	137.0 ± 9.0	-	2.3 ± 0.1**
1,2,4-Trichlorobenzene	0.0 (Controls)	166 ± 51	3.9 ± 0.2
	0.018 ± 0.003	151 ± 60	4.2 ± 0.2
	0.039 ± 0.005	159 ± 38	3.9 ± 0.1
	0.079 ± 0.011	157 ± 25	3.7 ± 0.1
	0.162 ± 0.028	125 ± 27	3.6 ± 0.5
	0.363 ± 0.056	107 ± 30	3.6 ± 0.2
	0.694 ± 0.140	32 ± 20**	3.0 ± 0.2**
1,3-Dichlorobenzene	0.0 (Controls)	165 ± 23	4.2 ± 0.1
	0.044 ± 0.012	167 ± 34	4.4 ± 0.1
	0.102 ± 0.023	178 ± 30	4.3 ± 0.1
	0.182 ± 0.039	212 ± 37	4.5 ± 0.2
	0.373 ± 0.053	137 ± 46	4.1 ± 0.2
	0.689 ± 0.156	190 ± 39	4.3 ± 0.2
	1.45 ± 0.28	93 ± 30**	3.5 ± 0.2**

TABLE 11 Cont. MEAN EXPOSURE CONCENTRATIONS OF SELECTED TEST CHEMICALS AND EFFECTS UPON REPRODUCTIVE SUCCESS AND GROWTH IN DAPHNIA MAGNA DURING 28 DAY CHRONIC TESTS

Compound	Chemical Concentration mg·L^{-1} ($\bar{x}$ ± s.d.)	Number of Young Produced Per Adult ($\bar{x}$ ± s.d.)	Length (mm) of Adults ($\bar{x}$ ± s.d.)
Tetrachloroethylene	0.0 (Controls)	154 ± 47	3.9 ± 0.2
	0.75 ± 0.036	165 ± 45	4.1 ± 0.2
	0.159 ± 0.085	111 ± 76	3.9 ± 0.4
	0.254 ± 0.094	169 ± 46	4.0 ± 0.2
	0.505 ± 0.250	169 ± 43	4.1 ± 0.1
	1.11 ± 0.48	58 ± 26**	3.6 ± 0.1**
	1.75 ± 1.10	0	0
Arsenic^{+3}	0.0 (Controls)	83 ± 28	3.6 ± 0.2
	0.073 ± 0.006	126 ± 27	3.7 ± 0.2
	0.132 ± 0.004	81 ± 31	3.6 ± 0.2
	0.270 ± 0.014	115 ± 19	3.7 ± 0.2
	0.633 ± 0.034	132 ± 10	3.7 ± 0.3
	1.32 ± 0.03	0**	3.2 ± 0.3**
	2.68 ± 0.06	0**	3.2 a/

* Significantly different from controls ($p<0.05$).

** Significantly different from controls ($p<0.01$).

a/ Value for one animal only.

ethylene (1.11 $mg \cdot L^{-1}$), and arsenic^{+3} (1.32 $mg \cdot L^{-1}$). Length of adults was significantly ($p \leq 0.05$ or $p \leq 0.01$) less than that of controls at or above the following mean concentrations (Table 11): 1,1,2,2-tetrachloroethane (no data), 1,1,2-trichloroethane (26.0 $mg \cdot L^{-1}$), 1,2-dichloroethane (71.7 $mg \cdot L^{-1}$), 1,2,4-trichlorobenzene (0.694 $mg \cdot L^{-1}$), 1,3-dichlorobenzene (1.45 $mg \cdot L^{-1}$), tetrachloroethylene (1.11 $mg \cdot L^{-1}$), and arsenic^{+3} (1.32 $mg \cdot L^{-1}$). "No effect" concentration ranges for these chemicals were: 1,1,2,2-tetrachloroethane (6.85-14.4 $mg \cdot L^{-1}$), 1,1,2-trichloroethane (13.2-26.0 $mg \cdot L^{-1}$), 1,2-dichloroethane (10.6-20.7 $mg \cdot L^{-1}$), 1,2,4-trichlorobenzene (0.363-0.694 $mg \cdot L^{-1}$), 1,3-dichlorobenzene (0.689-1.45 $mg \cdot L^{-1}$), tetrachloroethylene (1.11-1.75 $mg \cdot L^{-1}$), and arsenic^{+3} (0.63-1.32 $mg \cdot L^{-1}$).

Fathead Minnows - Arsenic^{+3} did not adversely affect fathead minnow hatching success, nor the percentage of newly hatched fry that died immediately after hatch at the exposure concentrations tested (Table 12). Arsenic^{+3} exposures did not result in any abnormal morphological characteristics of the fry. Survival of the fish through 24 days post-hatch was significantly ($p \leq 0.01$) reduced at the highest exposure concentration (16.5 $mg \cdot L^{-1}$). Wet weight and length of the fish at 24 days post-hatch were both significantly ($p \leq 0.01$) reduced at concentrations of 4.30 $mg \cdot L^{-1}$ and above. The "no-effect" concentration was between 2.13 and 4.30 $mg \cdot L^{-1}$.

Mercury^{+2} did not affect hatching success nor the percentage of fry that died immediately after hatch at the exposure concentrations tested (Table 13). The two highest exposures (1.85 and 3.70 $\mu g \cdot L^{-1}$) resulted in significantly greater ($p \leq 0.01$) percentages of fry with gross deformities, mainly in the form of spinal curvatures; and in reduced survival of juvenile fish through 30 days post-hatch. All exposures resulted in significant ($p \leq 0.01$) reductions in weight and length at test termination. Therefore, the "no effect" concentra-

TABLE 12. HATCHABILITY, DEVELOPMENT, SURVIVAL AND GROWTH OF FATHEAD MINNOWS (*Pimephales promelas*) EXPOSED TO ARSENIC^{+3} ($NaAsO_2$) FOR 30 DAYS POST-FERTILIZATION

	Mean As^{+3} Concentration ($mg \cdot L^{-1}$)					
	Control 0.00 ± 0.00 (n=13)	1.06 ± 0.28 (n=18)	2.13 ± 0.39 (n=18)	4.30 ± 0.50 (n=18)	7.37 ± 0.49 (n=18)	16.5 ± 1.03 (n=18)
Mean % hatch	86.0	92.5	91.5	93.1	91.1	86.4
Mean % dead fry at transfer from egg cups	0.0	0.1	1.1	2.6	3.6	2.9
Mean % normal fry[a] at transfer from egg cups	97.5	94.7	94.9	95.2	92.3	94.9
Mean % survival of fish at 24 days post-hatch	95.0	70.0	90.0	77.5	97.5	22.5
Mean fry wet wt (g) at 24 days post-hatch	0.058	0.056	0.050	0.041**	0.026**	0.012**
Mean fry length (mm) at 24 days post-hatch	17.0	17.0	16.3	15.6**	13.3**	11.2**

[a] Fry were considered abnormal when they exhibitied gross morphological anomalies such as spinal curvatures, or were dead after hatching.

** Significantly different from controls ($p \leq 0.01$).

TABLE 13. HATCHABILITY, DEVELOPMENT, SURVIVAL AND GROWTH OF FATHEAD MINNOWS (*Pimephales promelas*) EXPOSED TO INORGANIC MERCURY ($HgCl_2$) FOR 35 DAYS POST-FERTILIZATION

	Mean Hg^{+2} Concentration ($\mu g \cdot L^{-1}$)					
	Control 0.01 ± 0.01 (n=22)	0.23 ± 0.03 (n=22)	0.48 ± 0.07 (n=22)	0.87 ± 0.08 (n=22)	1.85 ± 0.2 (n=22)	3.70 ± 0.6 (n=21)
Mean % hatch[a]	67.3	73.2	69.6	75.8	77.1	63.1
Mean % dead fry at transfer[a] from egg cups	4.3	4.7	4.1	3.8	2.8	5.4
Mean % normal fry[b] at transfer[a] from egg cups	100.0	100.0	100.0	100.0	25.0**	0.0**
Mean % survival of fish at 30 days post-hatch[a]	100.0	100.0	100.0	100.0[c]	56.7**[c]	11.6**[c]
Mean fish wet wt (g) at 30 days post-hatch	0.215	0.193**	0.193**	-[c]	-[c]	0.013**
Mean fish length (mm) at 30 days post-hatch	22.4	21.5**	21.2**	19.9**[c]	17.2**[c]	9.4**

[a] Percentage values were subjected to arcsin transformation for statistical analysis.

[b] Abnormal fry had gross morphological anomalies such as spinal curvatures.

[c] All fish at the 0.87 and 1.85 $\mu g \cdot L^{-1}$ exposures died on day 29 post-hatch due to a diluter malfunction, and weight data were not available. Survival and length data were from 29 days post-hatch.

** Significantly different from controls ($p \leq 0.01$).

tion was less than 0.23 $\mu g \cdot L^{-1}$, the lowest exposure concentration.

Flagfish - The early life-stage tests were conducted with arsenic^{+3} and flagfish. None of the parameters were significantly affected in the first test at mean As^{+3} concentrations of 0.30, 0.60, 1.20, 2.34, and 5.04 $mg \cdot L^{-1}$. However, the weight of juvenile fish at the end of the exposure period at the exposure concentration of 5.04 $mg \cdot L^{-1}$ was considerably less ($\bar{x}$ = 0.118 g) than the weight of control fish ($\bar{x}$ = 0.135 g). Arsenic^{+3} did not adversely affect hatching success at any of the concentrations tested (Table 14). A significant ($p \leq 0.05$) increase in hatching success was observed at a concentration of 4.12 $mg \cdot L^{-1}$, but higher exposures did not result in significant changes. There were no significant ($p \geq 0.05$) differences from controls in the percentages of dead fry or normal fry at the time of transfer from the egg cups, nor in the survival of fish through 25 days post-hatch. Wet weight was significantly ($p \leq 0.01$) reduced at concentrations of 7.57 $mg \cdot L^{-1}$ and above. Length of the fish was significantly ($p \leq 0.05$) reduced at concentrations of 4.12 $mg \cdot L^{-1}$ and above. The "no-effect" concentration was between 2.13 and 4.12 $mg \cdot L^{-1}$.

Di-n-butylphthalate Uptake, Elimination and Metabolism by Fish

^{14}C-labeled di-*n*-butylphthalate was rapidly taken up from the water and accumulated in the tissues of fathead minnows (Fig. 1). A steady-state level of total ^{14}C was attained within 4 hrs of exposure. This steady-state level was maintained, with some fluctuations, throughout the 264 hr (11 day) exposure. The mean bioconcentration factor (BCF) in ^{14}C equivalents of di-*n*-butylphthalate for the exposure period from 4 hr to 11 days with the lower exposure was 2,068. The mean BCF for the higher exposure between 4 and 120 hr was 2,125. The time interval for determining the BCF at steady-state in the higher exposure was not considered beyond 120 hr due to a decrease in exposure

TABLE 14. HATCHABILITY, DEVELOPMENT, SURVIVAL AND GROWTH OF FLAGFISH (Jordanella floridae) EXPOSED TO ARSENIC^{+3} ($NaAsO_2$) FOR 30 DAYS POST-FERTILIZATION

		Mean As^{+3} Concentration ($mg \cdot L^{-1}$)				
	Control 0.00 ± 0.00 (n=17)	1.21 ± 0.35 (n=20)	2.13 ± 0.38 (n=20)	4.12 ± 0.29 (n=20)	7.57 ± 0.61 (n=20)	16.3 ± 0.85 (n=20)
Mean % hatch	56.4	66.0	64.4	87.4*	78.3	76.1
Mean % dead fry at transfer from egg cups	2.2	2.2	1.4	0.0	0.9	1.0
Mean % normal[a/] fry at transfer from egg cups	95.6	96.5	97.2	98.3	99.1	99.0
Mean % survival of fish at 25 days post-hatch	77.5	85.0	80.0	95.0	82.5	75.0
Mean fry wet wt (g) at 25 days post-hatch	0.056	0.043	0.055	0.046	0.031**	0.031**
Mean fry length (mm) at 25 days post-hatch	13.2	12.6	12.7	12.1*	10.8**	7.9**

a/ Fry were considered abnormal when they exhibited gross morphological anomalies such as spinal curvatures, or were dead after hatching.

* Significantly different from controls ($p \leq 0.05$).

** Significantly different from controls ($p \leq 0.01$).

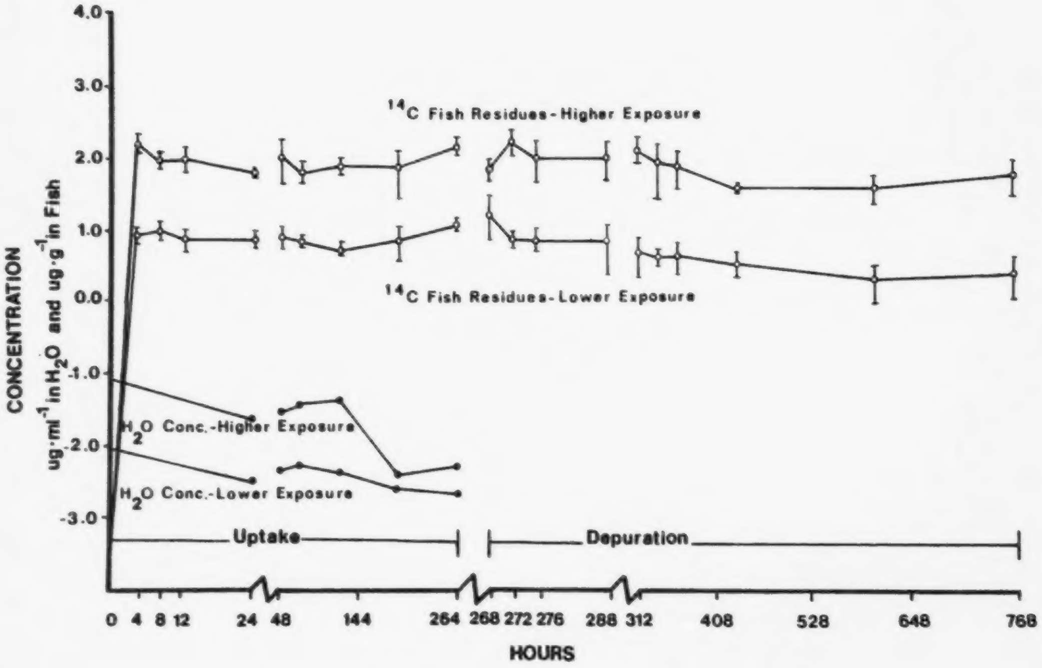

Figure 1. Log mean exposure water concentrations (μg·mL^{-1}) and log mean concentrations (± S.D.) of whole body ^{14}C equivalents (μg·g^{-1}) of di-*n*-butylphthalate in fathead minnows (*Pimephales promelas*) exposed for 11 days (264 hrs) and allowed to depurate 21 days (500 hrs).

concentrations beyond 120 hr and the slow depuration rate.

^{14}C residues were eliminated slowly in the fish tissues upon transfer to clean flowing water. The half-lives ($t_{\frac{1}{2}}$) for total ^{14}C residues in fish tissue were 216 hr (9 days) and 243 hr (10 days) for lower and higher exposure concentrations, respectively.

After 1 and 3 days of exposure, di-n-butylphthalate contributed 21.6 and 53.0%, respectively, of the total radioactivity measured in the fish. These percentages were determined from the percentages of supernatant radioactivity that were due to di-n-butylphthalate (Table 15). After 11 days of exposure, di-n-butylphthalate contributed only 8.1% of the total radioactivity. Application of the mean of these three percentages (27.6%) gave di-n-butylphthalate BCF values of 570 and 590 for lower and higher exposures, respectively.

After 1 day of exposure, 7 radioactive spots were present on the TLC plate. No phthalic acid was detected. After 3 days of exposure, 8 radioactive spots were observed on the TLC plate, with 1.9% of the radioactivity contributed by the metabolite phthalic acid. Only 4.4% of the radioactivity remained at the TLC plate origin, while 79.3% had the same R_f as di-n-butylphthalate. After 11 days of exposure, 8 radioactive spots were present, with 26.0% of the radioactivity remaining at the origin, 2.2% as phthalic acid, and only 14.6% as di-n-butylphthalate. On day 11, the percentage of total radioactivity associated with the pellet increased to 44.4%.

Di-n-butylphthalate Binding

Di-n-butylphthalate was rapidly converted by rainbow trout liver microsomes to unextractable compound present in the aqueous phase (Table 16). Approximately 40% of the radiolabeled compound was converted within 15 min, and 60% within 1 hr. Irreversible protein binding occurred throughout the

TABLE 15. DISTRIBUTION OF RADIOACTIVITY IN FATHEAD MINNOWS (*Pimephales promelas*) EXPOSED TO ^{14}C-DI-*n*-BUTYLPHTHALATE

Exposure Duration (Days)	Supernatant			Pellet[b]
	% of Total ^{14}C	% of ^{14}C as Di-*n*-butylphthalate[a]	% of ^{14}C as Phthalic Acid[a]	% of Total ^{14}C
1	66.9	32.3	0	33.1
2	61.2	n.d.[c]	n.d.	38.8
3	66.9	79.3	1.9	33.1
11	55.6	14.6	2.2	44.4

[a] Percentages of supernatant ^{14}C contributed by di-*n*-butylphthalate and phthalic acid were determined by thin-layer chromatography, autoradiography, and scintillation counting techniques.

[b] Pellet produced by centrifugation of fish tissue homogenate at 2,150 x g for 20 min.

[c] Not determined.

TABLE 16. DISTRIBUTION (% ± S.D.) OF ^{14}C AFTER INCUBATION OF ^{14}C-DI-*n*-BUTYLPHTHALATE FOR VARIOUS TIME INTERVALS WITH MICROSOMAL FRACTIONS OF RAINBOW TROUT (*Salmo gairdneri*) LIVER AND POST-MITOCHONDRIAL SUPERNATANT OF *DAPHNIA MAGNA*. (Values are the Means of Three Separately Prepared Tissue Fractions)

Time (Min)	Hexane Extracted[a]	Aqueous or Unextracted in Hexane[b]	Protein Bound[b]	Total % Recovery[c]
		Rainbow Trout		
0	90.3 ± 1.2	7.7 ± 1.1	2.0 ± 0.3	90.8 ± 4.9
15	54.8 ± 4.3	38.1 ± 3.7	7.0 ± 1.0	91.5 ± 5.9
30	44.3 ± 6.7	46.3 ± 3.0	8.7 ± 3.1	95.4 ± 2.3
45	34.2 ± 2.5	57.3 ± 2.8	8.5 ± 1.7	95.1 ± 4.7
60	32.7 ± 0.6	57.7 ± 1.5	9.6 ± 1.9	94.0 ± 5.5
120	27.5 ± 1.3	63.1 ± 2.8	9.4 ± 1.8	91.2 ± 5.5
		Daphnia		
0	97.9 ± 0.7	1.9 ± 0.7	0.22 ± 0.07	86.3 ± 12.5
15	94.7 ± 0.5	4.7 ± 0.7	0.54 ± 0.39	87.1 ± 3.6
30	92.4 ± 1.7	7.0 ± 1.7	0.57 ± 0.32	87.0 ± 14.4
45	93.2 ± 1.8	6.2 ± 1.8	0.55 ± 0.09	89.7 ± 3.0
60	88.2 ± 6.7	11.3 ± 6.1	0.48 ± 0.47	78.6 ± 13.9

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[a] Percent of total added ^{14}C in 0.1 mL solution which was recovered in hexane after extraction of reaction mixture.

[b] Percent dpm in aqueous fraction (soluble) and protein pellet relative to dpm extractable in hexane.

[c] Total percent recovery is based on dpm recoverable in all fractions divided by total ^{14}C in the reaction mixture.

2 hr incubation with ^{14}C labeled di-*n*-butylphthalate. Approximately 9% of the radiolabeled compound was bound to the trout liver microsomes during a 2 hr incubation with 7% of the total bound in the first 15 min of incubation.

Radiolabeled di-*n*-butylphthalate was converted more slowly by *Daphnia* PMS during a 1 hr incubation than by trout liver microsomes. Only about 10% of the radiolabeled compound was converted (Table 16) to water soluble hexane unextractable compounds. Less than 1% of the di-*n*-butylphthalate was irreversibly bound to protein.

Microsomal Metabolism and Binding of Chlorinated Hydrocarbons by Rainbow Trout and Daphnia

Measurements were made of the distribution of radioactivity after ^{14}C-labeled carbon tetrachloride was incubated with rainbow trout liver microsomes and *Daphnia* PMS tissue fractions. Most of these compounds were readily volatilized from the reaction mixture in spite of a silica gel trap. This resulted in low recoveries of the compound at the termination of chemical reaction. Most of the radioactivity was present in the hexane extract at each incubation time interval (Table 17). This indicated that most of the radioactivity remained as parent carbon tetrachloride. However, the amount recoverable with hexane decreased with incubation time. Radioactivity in the aqueous phase and the CO_2 trap increased with concomitant decrease of the hexane-extracted radiocarbon. Carbon tetrachloride bound slowly with the microsomal protein fractions of rainbow trout liver and *Daphnia* PMS. Formation of the aqueous metabolites and the protein binding of carbon tetrachloride did not appear to be linear with time of incubation. Both species were capable of slowly metabolizing this hepatoxin *in vitro* via microsomal mixed function oxidases.

TABLE 17. DISTRIBUTION (% ± S.D.) OF ^{14}C AFTER INCUBATION OF ^{14}C-CARBON TETRACHLORIDE FOR VARIOUS TIME INTERVALS WITH MICROSOMAL FRACTIONS OF RAINBOW TROUT (*Salmo gairdneri*) LIVER AND POST-MITOCHONDRIAL SUPERNATANT OF *DAPHNIA MAGNA*. (Values are the Means of Three Separately Prepared Tissue Fractions)

Time (Min)	Hexane[a] Extracted	Aqueous or[b] Unextracted in Hexane	CO_2[c] Trap	Protein[b] Bound	Total %[d] Recovery
			Rainbow Trout		
0	91.0 ± 5.8	0.56 ± 0.15	0.012 ± 0.016	0.26 ± 0.10	61.8 ± 11.9
15	92.6 ± 4.2	0.69 ± 0.13	0.15 ± 0.13	0.35 ± 0.10	52.7 ± 8.9
30	88.1 ± 3.0	0.73 ± 0.15	0.097 ± 0.095	0.37 ± 0.15	47.9 ± 16.8
45	90.1 ± 0.8	0.89 ± 0.05	0.15 ± 0.15	0.53 ± 0.13	45.6 ± 7.4
60	87.1 ± 1.8	1.20 ± 0.28	0.25 ± 0.21	0.61 ± 0.30	37.5 ± 8.3
120	84.7 ± 5.9	1.10 ± 0.16	0.18 ± 0.20	0.60 ± 0.15	40.0 ± 4.7
			Daphnia		
0	95.9 ± 1.8	0.72 ± 0.43	0.09 ± 0.02	0.061 ± 0.03	55.8 ± 16.2
15	91.4 ± 1.3	0.99 ± 0.38	0.15 ± 0.14	0.074 ± 0.03	45.9 ± 9.3
30	93.5 ± 2.3	0.91 ± 0.24	0.06 ± 0.08	0.12 ± 0.10	47.1 ± 4.7
45	89.1 ± 1.2	1.30 ± 0.21	0.38 ± 0.13	0.10 ± 0.06	38.3 ± 3.6
60	87.5 ± 0.9	1.50 ± 0.56	0.13 ± 0.18	0.09 ± 0.10	37.5 ± 2.9

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[a] Percent of total added dpm in 0.1 mL solution which could be recovered in hexane after the extraction of reaction mixture.

[b] Percent dpm in aqueous fraction (soluble and protein pellet) relative to dpm extractable in hexane.

[c] Percent radioactivity trapped in Carbosorb II® relative to dpm extractable in hexane.

[d] Total percent recovery is based on the dpms recoverable in all fractions including the silica gel trap divided by the total added dpm in the reaction mixture.

Chloroform was rapidly converted to hexane-unextractable water soluble metabolites in rainbow trout liver microsomes and Daphnia PMS (Table 18). Approximately 40% of the radioactivity was found in the aqueous phase and only 53% was extracted in hexane within 1 min of incubation with rainbow trout liver microsomes. Similarly, the aqueous phase from Daphnia contained more than 50% of the radioactivity as compared to 25-40% in the hexane extract. Radioactivity in the aqueous phase increased with incubation time to 70% in the case of Daphnia and to about 45% in rainbow trout. Measureable radioactivity was also found in the carbon dioxide traps of both animal species. Rainbow trout liver microsomes showed increased protein binding with incubation time. However, Daphnia showed little change in protein bound radioactivity with incubation time.

Most (87-94%) of the radioactivity spiked in the microsomal tissue preparation with ^{14}C chlorobenzene was extractable in hexane even after 120 min of incubation time with rainbow trout liver microsomes and 60 min with Daphnia PMS (Table 19). The aqueous phase of the reaction mixture, in both species, showed small percentages (0.6-2.3) of water soluble products of metabolism. Higher amounts of protein bound radioactivity were found with rainbow trout liver than with Daphnia tissue preparations.

Rainbow trout appeared to show higher metabolic activity than Daphnia for 1,1,2-trichloroethylene and 1,1,2-trichloroethane (Tables 20 and 21). More polar metabolites of 1,1,2-trichloroethylene and 1,1,2-trichloroethane were formed by rainbow trout liver microsomes than Daphnia PMS. Trichloroethane was more readily converted to water soluble products than trichloroethylene in the case of rainbow trout. On the other hand, Daphnia converted both of the compounds to aqueous metabolites at similar rates, but at much slower rates than rainbow trout. Both compounds showed protein binding with

TABLE 18. DISTRIBUTION (% ± S.D.) OF ^{14}C AFTER INCUBATION OF ^{14}C-CHLOROFORM FOR VARIOUS TIME INTERVALS WITH MICROSOMAL FRACTIONS OF RAINBOW TROUT *(Salmo gairdneri)* LIVER AND POST-MITOCHONDRIAL SUPERNATANT OF *DAPHNIA MAGNA*. (Values are the Means of Three Separately Prepared Tissue Fractions)

Time (Min)	Hexane[a] Extracted	Aqueous or[b] Unextracted in Hexane	CO_2[c] Trap	Protein[b] Bound	Total %[d] Recovery
			Rainbow Trout		
0	53.2 ± 25.3	38.6 ± 25.8	0.25 ± 0.32	1.1 ± 0.6	87.9 ± 25.3
15	45.9 ± 27.2	43.4 ± 27.5	0.09 ± 0.04	4.2 ± 2.6	86.8 ± 14.0
30	44.3 ± 20.7	44.3 ± 28.7	0.19 ± 0.23	3.5 ± 3.2	91.4 ± 15.7
45	46.3 ± 25.9	44.8 ± 26.8	0.18 ± 0.29	4.6 ± 1.7	83.6 ± 12.5
60	42.1 ± 27.7	44.8 ± 26.3	1.2 ± 1.7	4.6 ± 2.0	81.9 ± 14.9
120	45.3 ± 16.7	42.4 ± 17.5	0.73 ± 1.0	5.9 ± 4.2	83.7 ± 11.6
			Daphnia		
0	39.7 ± 23.4	54.0 ± 25.9	2.4 ± 3.8	1.6 ± 2.4	83.0 ± 14.1
15	40.3 ± 20.7	53.0 ± 22.8	2.3 ± 3.5	1.5 ± 2.5	77.0 ± 14.8
30	37.8 ± 22.8	56.1 ± 23.9	1.7 ± 2.1	1.3 ± 1.9	85.8 ± 11.5
45	25.4 ± 7.3	70.1 ± 6.5	0.52 ± 0.54	1.5 ± 2.0	73.3 ± 28.8
60	26.2 ± 15.4	69.3 ± 16.5	0.11 ± 0.12	1.5 ± 1.5	74.9 ± 14.2

[a] Percent of total added dpm in 0.1 mL solution which could be recovered in hexane after the extraction of reaction mixture.

[b] Percent dpm in aqueous fraction (soluble and protein pellet) relative to dpm extractable in hexane.

[c] Percent radioactivity trapped in Carbosorb II® relative to dpm extractable in hexane.

[d] Total percent recovery is based on the dpms recoverable in all fractions including the silica gel trap divided by the total added dpm in the reaction mixture.

TABLE 19. DISTRIBUTION (% ± S.D.) OF ^{14}C AFTER INCUBATION OF ^{14}C-CHLOROBENZENE FOR VARIOUS TIME INTERVALS WITH MICROSOMAL FRACTIONS OF RAINBOW TROUT (*Salmo gairdneri*) LIVER AND POST-MITOCHONDRIAL SUPERNATANT OF *DAPHNIA MAGNA*. (Values are the Means of Three Separately Prepared Tissue Fractions)

Time (Min)	Hexane[a] Extracted	Aqueous or[b] Unextracted in Hexane	CO_2[c] Trap	Protein[b] Bound	Total %[d] Recovery
			Rainbow Trout		
0	92.2 ± 2.3	0.56 ± 0.06	0.006 ± 0.004	0.4 ± 0.1	75.2 ± 13.4
15	94.4 ± 1.8	0.95 ± 0.15	0.03 ± 0.01	0.60 ± 0.27	58.4 ± 8.3
30	92.5 ± 1.2	1.0 ± 0.3	0.015 ± 0.009	0.79 ± 0.17	50.0 ± 8.3
45	93.4 ± 2.1	1.3 ± 0.2	0.070 ± 0.014	0.8 ± 0.0	42.6 ± 2.5
60	92.3 ± 2.6	1.5 ± 0.2	0.014 ± 0.010	0.56 ± 0.40	39.1 ± 6.6
120	86.9 ± 1.0	1.9 ± 0.2	0.21 ± 0.25	1.2 ± 0.70	29.2 ± 3.5
			Daphnia		
0	91.2 ± 4.0	1.2 ± 0.17	0.060 ± 0.004	0.06 ± 0.026	66.4 ± 15.6
15	94.4 ± 2.0	1.7 ± 0.15	0.024 ± 0.006	0.13 ± 0.08	49.1 ± 8.4
30	92.5 ± 2.7	2.1 ± 0.06	0.042 ± 0.030	0.11 ± 0.08	41.3 ± 16.6
45	90.6 ± 2.1	2.1 ± 0.07	0.070 ± 0.014	0.15 ± 0.02	33.3 ± 11.2
60	92.5 ± 2.7	2.3 ± 0.36	0.090 ± 0.010	0.053 ± 0.006	35.7 ± 6.1

[a] Percent of total added dpm in 0.1 mL solution which could be recovered in hexane after the extraction of reaction mixture.

[b] Percent dpm in aqueous fraction (soluble and protein pellet) relative to dpm extractable in hexane.

[c] Percent radioactivity trapped in Carbosorb II® relative to dpm extractable in hexane.

[d] Total percent recovery is based on the dpms recoverable in all fractions including the silica gel trap divided by the total added dpm in the reaction mixture.

TABLE 20. DISTRIBUTION (% ± S.D.) OF ^{14}C AFTER INCUBATION OF ^{14}C-1,1,2-TRICHLOROETHYLENE FOR VARIOUS TIME INTERVALS WITH MICROSOMAL FRACTIONS OF RAINBOW TROUT (*Salmo gairdneri*) LIVER AND POST-MITOCHONDRIAL SUPERNATANT OF *DAPHNIA MAGNA*. (Values are the Means of Three Separately Prepared Tissue Fractions)

Time (Min)	Hexane[a] Extracted	Aqueous or[b] Unextracted in Hexane	CO_2[c] Trap	Protein[b] Bound	Total %[d] Recovery
			Rainbow Trout		
0	89.0 ± 3.4	1.1 ± 0.11	0.032 ± 0.007	0.09 ± 0.01	63.3 ± 15.9
15	92.2 ± 4.9	1.6 ± 0.06	0.096 ± 0.090	0.08 ± 0.08	54.0 ± 9.2
30	84.5 ± 5.8	6.3 ± 7.5	0.063 ± 0.046	0.4 ± 0.5	49.2 ± 11.9
45	88.3 ± 3.5	2.2 ± 0.15	0.21 ± 0.27	0.16 ± 0.09	39.9 ± 4.4
60	85.4 ± 3.6	1.8 ± 0.35	0.11 ± 0.11	0.14 ± 0.05	46.5 ± 8.2
120	82.8 ± 3.2	2.6 ± 0.80	0.19 ± 0.10	0.31 ± 0.20	32.7 ± 5.9
			Daphnia		
0	88.8 ± 2.1	1.03 ± 0.24	0.06 ± 0.01	0.024 ± 0.032	54.6 ± 0.6
15	89.4 ± 1.7	1.56 ± 0.16	0.10 ± 0.04	0.023 ± 0.017	42.4 ± 4.1
30	90.5 ± 3.7	1.8 ± 0.23	0.14 ± 0.09	0.020 ± 0.014	42.7 ± 5.5
45	91.5 ± 5.0	1.9 ± 0.42	0.03 ± 0.01	0.013 ± 0.011	37.4 ± 0.6
60	89.1 ± 1.3	1.95 ± 0.64	0.10 ± 0.01	0.012 ± 0.011	34.5 ± 3.2

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[a] Percent of total added dpm in 0.1 mL solution which could be recovered in hexane after the extraction of reaction mixture.

[b] Percent dpm in aqueous fraction (soluble and protein pellet) relative to dpm extractable in hexane.

[c] Percent radioactivity trapped in Carbosorb II® relative to dpm extractable in hexane.

[d] Total percent recovery is based on the dpms recoverable in all fractions including the silica gel trap divided by the total added dpm in the reaction mixture.

TABLE 21. DISTRIBUTION (% ± S.D.) OF ^{14}C AFTER INCUBATION OF ^{14}C-1,1,2-TRICHLOROETHANE FOR VARIOUS TIME INTERVALS WITH MICROSOMAL FRACTIONS OF RAINBOW TROUT (*Salmo gairdneri*) LIVER AND POST-MITOCHONDRIAL SUPERNATANT OF *DAPHNIA MAGNA*. (Values are the Means of Three Separately Prepared Tissue Fractions)

Time (Min)	Hexane[a] Extracted	Aqueous or[b] Unextracted in Hexane	CO_2[c] Trap	Protein[b] Bound	Total %[d] Recovery
			Rainbow Trout		
0	81.5 ± 24.7	12.4 ± 20.3	0.22 ± 0.35	0.65 ± 0.91	77.6 ± 21.7
15	79.3 ± 31.3	16.7 ± 27.4	0.049 ± 0.07	1.6 ± 2.5	75.6 ± 18.6
30	76.3 ± 34.1	15.1 ± 24.5	0.67 ± 0.25	1.3 ± 2.1	68.9 ± 16.3
45	77.8 ± 28.7	15.8 ± 25.6	0.18 ± 0.28	1.46 ± 2.30	69.4 ± 13.3
60	77.9 ± 31.7	15.5 ± 24.9	1.1 ± 1.8	1.47 ± 2.20	66.7 ± 11.1
120	76.4 ± 30.9	16.8 ± 26.7	0.70 ± 1.00	1.26 ± 1.50	59.7 ± 9.6
			Daphnia		
0	96.3 ± 0.6	0.77 ± 0.30	0.005 ± 0.004	0.004 ± 0.004	71.0 ± 14.7
15	97.2 ± 1.0	0.96 ± 0.30	0.007 ± 0.005	0.012 ± 0.008	60.1 ± 19.9
30	96.8 ± 0.8	1.1 ± 0.4	0.036 ± 0.029	0.009 ± 0.001	49.6 ± 7.6
45	97.0 ± 0.8	1.1 ± 0.14	0.015 ± 0.007	0.010 ± 0.014	47.3 ± 13.4
60	97.0 ± 0.0	0.98 ± 0.04	0.02 ± 0.00	0.012 ± 0.011	50.0 ± 18.8

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a/ Percent of total added dpm in 0.1 mL solution which could be recovered in hexane after the extraction of reaction mixture.

b/ Percent dpm in aqueous fraction (soluble and protein pellet) relative to dpm extractable in hexane.

c/ Percent radioactivity trapped in Carbosorb II® relative to dpm extractable in hexane.

d/ Total percent recovery is based on the dpms recoverable in all fractions including the silica gel trap divided by the total added dpm in the reaction mixture.

rainbow trout or Daphnia microsomal mixed function oxidase system in vitro.

Both rainbow trout and Daphnia metabolized chloroform most readily and carbon tetrachloride least readily, based upon the percentages of total radioactivity present in the aqueous phase and in the protein bound phase. For the remaining three compounds, the orders for rate of metabolism were not the same between species. The order for rainbow trout was chloroform > 1,1,2-trichloroethane > 1,1,2-trichloroethylene > chlorobenzene > carbon tetrachloride. The order for Daphnia was chloroform > chlorobenzene > 1,1,2-trichloroethylene > 1,1,2-trichloroethane $\simeq$ carbon tetrachloride.

Mixed Function Oxidase Levels

Microsomal monooxygenase or mixed function oxidase assays of rainbow trout liver and Daphnia PMS fractions were performed. Rainbow trout liver microsomes had mean values of 0.28 and 0.19 $\text{nM}\cdot\text{mg}^{-1}$ of cytochrome P-450 and cytochrome b_5, respectively (Table 22). The level of NADPH cytochrome c reductase activity in rainbow trout liver microsomes was 16 nM of cytochrome c $\text{reduced}\cdot\text{min}^{-1}\cdot\text{mg}^{-1}$ protein. Rainbow trout liver microsomes metabolized aniline at a very slow rate of 0.04-0.05 $\text{nM}\cdot\text{mg}^{-1}$ $\text{protein}\cdot\text{min}^{-1}$ (Table 22). PMS from adult Daphnia showed a mean value of 42 ± 5.3 nM of cytochrome c reductase $\text{activity}\cdot\text{min}^{-1}\cdot\text{mg}^{-1}$ protein, which was higher than rainbow trout.

TABLE 22. MIXED FUNCTION OXIDASE SYSTEMS OF RAINBOW TROUT (*Salmo gairdneri*) LIVER AND *DAPHNIA MAGNA*

Enzymes	Rainbow Trout	Daphnia
Cytochrome[a] P-450	0.28 ± 0.1 (4)[d]	N.D.
Cytochrome b_5[a]	0.19 ± 0.05 (4)	N.D.
NADPH Cytochrome[b] c-reductase	15.9 ± 2.2 (8)	42 ± 5.3 (3)
Aniline hydroxylase[c]	0.05 ± 0.01 (3)	-

[a] Nanomoles·mg^{-1} microsomal protein ± S.D.

[b] Nanomoles of cytochrome c reduced·min^{-1}·mg^{-1} protein ± S.D.

[c] Nanomoles of *p*-aminophenol formed·min^{-1}·mg^{-1} protein ± S.D.

[d] Numbers in parentheses are the number of tissue preparations from separate animal batches.

SECTION VI

DISCUSSION

EPA PRIORITY POLLUTANTS

Chlorinated Ethanes. In this study, the acute toxicities of a series of chlorinated ethanes from 1,2-dichloroethane to hexachloroethane were determined with Daphnia magna (Table 5). Chronic toxicity to Daphnia was determined for 1,2-dichloroethane, 1,1,2-trichloroethane, and 1,1,2,2-tetrachloroethane (Table 11). The acute toxicity of hexachloroethane was also determined for rainbow trout and midges (Tables 2 and 7).

Toxicity to Daphnia increased with degree of chlorination in acute and chronic tests. This direct relationship between toxicity and degree of chlorination has also been noted with fathead minnows (U.S. EPA, 1980b) and bluegill sunfish (Buccafusco et al., 1981). However, this clear relationship was not evident with Daphnia magna in a study by LeBlanc (1980).

In the present study, 48 hr LC_{50} values for Daphnia ranged from 268 $mg \cdot L^{-1}$ for 1,2-dichloroethane to 2.90 $mg \cdot L^{-1}$ for hexachloroethane. Comparison of our results with those of LeBlanc (1980) show good agreement of LC_{50} values for 1,2-dichloroethane (268 vs. 218 $mg \cdot L^{-1}$), but poor agreement for the remainder of the series from tri-through hexachloroethane. In a similar study of Adema (1978), LC_{50} values based on nominal concentrations of 1,1,2-trichloroethane were 43 $mg \cdot L^{-1}$ for fed and unfed Daphnia magna, which were about one-fourth the values reported here. In agreement with our acute test results, feeding did not affect the toxicity of this compound. Feeding did not appear to greatly affect the toxicity of any of the chlorinated ethanes tested here.

The 96 hr rainbow trout LC_{50} for hexachloroethane of 0.982 $mg \cdot L^{-1}$ was almost identical to the value of 0.980 for bluegill sunfish (Buccafusco et al., 1981) and somewhat lower than the LC_{50} of 1.53 $mg \cdot L^{-1}$ for fathead minnows (U.S. EPA, 1980b). A 48 hr LC_{50} of 5.85 $mg \cdot L^{-1}$ for the midge indicated that Tanytarsus was less sensitive to hexachloroethane in acute exposures than Daphnia magna, rainbow trout, or bluegill sunfish.

"No-effect" concentration ranges from the Daphnia magna chronic exposures were: 1,2-dichloroethane - 10.6 - 20.7 $mg \cdot L^{-1}$, 1,1,2-trichloroethane - 26.0 - 41.8 $mg \cdot L^{-1}$ and 1,1,2,2-tetrachloroethane - 6.85 - 14.4 $mg \cdot L^{-1}$ (Table 11). The chronic "no-effect" level for reproduction of 18 $mg \cdot L^{-1}$ for 1,1,2-trichloroethane by Adema (1978) was close to our value of 26.0 $mg.L^{-1}$. Similar to our work, Adema (1978) used completely filled and closed flasks to minimize the loss of volatile compounds. These same compounds have been studied in early life-stage exposures with the fathead minnow (U.S. EPA, 1980b). "No-effect" concentration ranges for fathead minnows were: 1,2-dichloroethane - 14.0 - 29.0 $mg \cdot L^{-1}$, 1,1,2-trichloroethane - 6.0 - 14.8 $mg.L^{-1}$, and 1,1,2,2-tetrachloroethane - 1.4 - 4.0 $mg \cdot L^{-1}$.

Tetrachloroethylene. The acute toxicity of tetrachloroethylene was determined with rainbow trout (Table 2), Daphnia magna (Table 5), and midges (Table 7). Tetrachloroethylene toxicity to rainbow trout was determined both with and without the carrier solvent, dimethylformamide (DMF).

Rainbow trout were the most sensitive of the three species and midges the least sensitive. The 96 hr LC_{50} of 4.99 $mg \cdot L^{-1}$ for tetrachloroethylene alone with rainbow trout was similar to the value of 5.84 $mg \cdot L^{-1}$ for tetrachloroethylene with DMF, and the 95% confidence intervals overlapped. Fish exposed to tetrachloroethylene with DMF were larger than those exposed to tetrachloroethylene alone, which may explain the slightly greater tolerance in the former test. Increased tolerance of rainbow trout to permethrin with an increase in body size was observed by Kumaraguru and Beamish (1981). The 96 hr LC_{50} values determined here for rainbow trout indicate that trout are more sensitive than other fish species tested. LC_{50} values of 13.5 and 18.4 $mg \cdot L^{-1}$ were obtained for fathead minnows in flow-through measured tests (U.S. EPA, 1980b; Alexander *et al.*, 1978), and 12.9 $mg \cdot L^{-1}$ for bluegill sunfish in a static unmeasured test (Buccafusco *et al.*, 1981).

A 48 hr LC_{50} value of 18.1 $mg \cdot L^{-1}$ for *Daphnia magna* in an unfed test was almost twice the value of 9.09 $mg \cdot L^{-1}$ in a fed test. This difference between results from fed and unfed tests was greatly reduced when the toxic responses were expressed as EC_{50} values. 48 Hr EC_{50} values of 8.50 and 7.49 $mg \cdot L^{-1}$ were obtained for unfed and fed tests, respectively. LeBlanc (1980) obtained a 48 hr LC_{50} of 17.7 $mg \cdot L^{-1}$ in a static unmeasured test.

Midges had a 48 hr LC_{50} of 30.8 $mg \cdot L^{-1}$. Of the five species of freshwater animals used in acute tests, midges were the most tolerant to tetrachloroethylene (U.S. EPA 1980c).

A 28 day chronic exposure of *Daphnia magna* to tetrachloroethylene resulted in a "no-effect" concentration range of 0.505 - 1.11 $mg \cdot L^{-1}$ (Table 11). Production of young and length of adult daphnids were both significantly reduced ($p<0.01$) at a mean exposure concentration of 1.11 $mg \cdot L^{-1}$. The "no-effect" range for *Daphnia* was very similar to the range of 0.5 - 1.4 $mg \cdot L^{-1}$ reported for the fathead minnow (U.S. EPA, 1980b).

Chlorinated Benzenes. Acute toxicity tests were conducted with rainbow trout for 1,2- and 1,4-dichlorobenzene, 1,2,4-trichlorobenzene, pentachlorobenzene with DMF as a carrier solvent, and hexachlorobenzene with DMF (Table 2). One acute test was conducted with bluegill sunfish and hexachlorobenzene with DMF (Table 3). *Daphnia magna* was tested in acute and chronic exposures to 1,3-dichlorobenzene and 1,2,4-trichlorobenzene (Tables 5 and 11). Midges were used in acute tests with 1,2-dichlorobenzene, 1,4-dichlorobenzene, and hexachlorobenzene with DMF (Table 7).

The 96 hr LC_{50} values for rainbow trout were 1.58 and 1.12 $mg \cdot L^{-1}$ for 1,2- and 1,4-dichlorobenzene, respectively. 1,4-Dichlorobenzene produced a 96 hr LC_{50} of 4.0 $mg \cdot L^{-1}$ in a flow-through measured test with the fathead minnow (U.S. EPA 1980b). Static unmeasured tests with bluegill sunfish and 1,2-dichlorobenzene gave 96 hr LC_{50}s of 27.0 $mg \cdot L^{-1}$ (Dawson *et al.*, 1977) and 5.6 $mg \cdot L^{-1}$ (Buccafusco *et al.*, 1981). A static unmeasured test with 1,4-dichlorobenzene and bluegills produced an LC_{50} of 4.3 $mg \cdot L^{-1}$ (Buccafusco *et al.*, 1981).

The 48 hr LC_{50} values for 1,3-dichlorobenzene and *Daphnia magna* in the present study were 7.43 and 7.23 $mg \cdot L^{-1}$ in unfed and fed tests, respectively. Feeding had virtually no effect upon the response. LeBlanc (1980) obtained an LC_{50} of 28 $mg \cdot L^{-1}$ in a static unmeasured test with this species.

The 48 hr LC_{50}s for midges exposed to 1,2- and 1,4-dichlorobenzene were 12.0 and 13.0 $mg \cdot L^{-1}$, respectively. *Daphnia magna* was the only other invertebrate species that had been tested with these compounds (U.S. EPA, 1980d) where LC_{50}s of 2.4 and 11 $mg \cdot L^{-1}$ were obtained in static unmeasured tests with 1,2- and 1,4-dichlorobenzene, respectively (LeBlanc, 1980).

1,2,4-Trichlorobenzene produced a 96 hr LC_{50} of 1.53 $mg \cdot L^{-1}$ in rainbow trout and 48 hr LC_{50}s of 2.09 and 1.68 in *Daphnia magna* for unfed and fed tests, respectively. A 96 hr LC_{50} of 2.87 $mg \cdot L^{-1}$ was reported with 1,2,4-trichlorobenzene and fathead minnows in a flow-through measured test (U.S. EPA, 1980b), and 3.4 $mg \cdot L^{-1}$ for bluegill sunfish in a static unmeasured test (Buccafusco _et al_., 1981). In a static unmeasured test with _Daphnia magna_, a 48 hr LC_{50} of 50 $mg \cdot L^{-1}$ was obtained (LeBlanc, 1980), a value about 24 times greater than ours.

Pentachlorobenzene (with DMF) did not yield sufficient mortalities in rainbow trout at 96 hr to allow for an estimation of the LC_{50}. However, by 144 hr sufficient mortalities had occurred to produce an LC_{50} estimate of 0.28 $mg \cdot L^{-1}$. Buccafusco _et al_ (1981) obtained a 96 hr LC_{50} of 0.25 $mg \cdot L^{-1}$ with bluegill sunfish in a static unmeasured test.

Hexachlorobenzene was insufficiently soluble in water to yield concentrations high enough to result in acute test mortalities and LC_{50} estimates. It was tested using the carrier solvent DMF with rainbow trout, bluegill sunfish and midges. The same observation was made in tests with fathead minnows (U.S. EPA, 1980b).

Chronic (28 day) exposure of _Daphnia magna_ to 1,3-dichlorobenzene resulted in a "no-effect" concentration range of 0.689 to 1.45 $mg \cdot L^{-1}$. Production of young and length of adults were both significantly reduced ($p<0.01$) at the highest exposure of 1.45 $mg \cdot L^{-1}$. The "no-effect" range for fathead minnows exposed to 1,3-dichlorobenzene in an early life-stage test was 1.0 to 2.3 $mg \cdot L^{-1}$ (U.S. EPA, 1980b).

Chronic exposure of _Daphnia magna_ to 1,2,4-trichlorobenzene resulted in a "no-effect" concentration range of 0.363 to 0.694 $mg \cdot L^{-1}$. Significant reductions ($p<0.01$) in both production of young and length of adults occurred at the highest exposure of 0.694 $mg \cdot L^{-1}$. The "no-effect" range for fathead minnows in an early life stage test was 0.499 to 0.995 $mg \cdot L^{-1}$ (U.S. EPA, 1980b).

A general trend of increased toxicity with increased degree of chlorination in the chlorinated benzenes had been noted (U.S. EPA, 1980e). In this study, 1,2,4-trichlorobenzene was more toxic to *Daphnia* than 1,3-dichlorobenzene in both acute and chronic tests. In the rainbow trout acute tests, pentachlorobenzene (144 hr LC_{50}) was more toxic than the di- and trichlorobenzenes. However, 1,2-di-, 1,4-di-, and 1,2,4-trichlorobenzene had quite similar LC_{50} values.

Hexachlorobutadiene. LC_{50} values (96 hr) of 0.320 and 0.324 $mg \cdot L^{-1}$ were determined for rainbow trout and bluegill sunfish, respectively (Tables 2 and 3). Only four other freshwater species have had acute values reported (U.S. EPA, 1980f; Leeuwangh *et al*., 1975). In a flow-through measured test, the fathead minnow had a 96 hr LC_{50} of 0.102 $mg \cdot L^{-1}$ (U.S. EPA, 1980b). LC_{50} values (96 hr) for the goldfish (*Carassius auratus*) and for the invertebrates *Asellus aquaticus* (Crustacea) and *Lymnaea stagnalis* (Mollusca) were 0.09, 0.13 and 0.21 $mg \cdot L^{-1}$, respectively, in measured static tests where the solutions were renewed daily (Leeuwangh *et al*., 1975).

Di-n-Butylphthalate. In this study a 48 hr LC_{50} of 3.70 $mg \cdot L^{-1}$ was obtained with *Daphnia magna* (Table 5). This value may be compared to 96 hr LC_{50} values of 2.10 $mg \cdot L^{-1}$ for the scud (*Gammarus pseudolimnaeus*) and >10.00 $mg \cdot L^{-1}$ for the crayfish (*Orconectes nais*) in static unmeasured tests (Mayer and Sanders, 1973). The fathead minnow, bluegill, channel catfish (*Ictalurus punctatus*), and rainbow trout had 96 hr LC_{50}s of 1.30, 0.73, 2.91, and 6.47 $mg \cdot L^{-1}$, respectively (Mayer and Sanders, 1973). Johnson and Finley (1980) reported the same values for these species except for rainbow trout for which they reported a 96 hr LC_{50} concentration of 2.6 $mg \cdot L^{-1}$. Sensitivities to di-*n*-butylphthalate have generally been similar amongst the different freshwater species tested (U.S. EPA, 1980g).

The mean bioconcentration factor (BCF) for di-*n*-butylphthalate (measured as ^{14}C equivalents) in whole body fathead minnows was ∿2,100. When expressed as parent compound alone, the mean BCF was 580. An equilibrium between the water and fish tissue was reached within 4 hr (Fig. 1). ^{14}C residues were eliminated very slowly (half-life = 9-10 days), indicating that much of the di-*n*-butylphthalate taken up was being metabolized and the ^{14}C retained.

Mayer and Sanders (1973) exposed four invertebrate species to ^{14}C di-*n*-butylphthalate. In *Daphnia magna* and *Gammarus pseudolimnaeus*, an equilibrium in ^{14}C residues between water and tissue was reached within 7 days. Tissue residues 400 and 1,350 times greater than water concentrations were obtained on day 7 for *Daphnia* and *Gammarus*, respectively. The midge, *Chironomus plumosus*, and the mayfly, *Hexagenia bilineata*, had residue concentration factors 720 and 430 times the water concentration on day 7 (last day of measurements), but an equilibrium was not evident. Following 7 days of exposure to ^{14}C di-*n*-butylphthalate, *Daphnia* transferred to fresh flowing water eliminated 50% of the total radioactivity after 3 days, but still retained 25% after 7 days (Mayer and Sanders, 1973).

Somewhat different results for di-*n*-butylphthalate accumulation of ^{14}C equivalents in the whole body were reported by Sanders *et al*. (1973). Tests with midge larvae (*Chironomus plumosus*), water flea (*Daphnia magna*), scud (*Gammarus pseudolimnaeus*), mayfly (*Hexagenia bilineata*), grass shrimp (*Palaemonetes kadiakensis*), and damselfly (*Ischnura verticalis*) showed biomagnification values at seven days of 6,600, 5,000, 6,500, 1,900, 5,000 (3 days) and 2,700, respectively. There did not seem to be further uptake between days 7 and 14 for the two species tested (water flea and scud) at this time interval.

Metabolism of di-n-butylphthalate has been measured in vitro using microsomal preparations from channel catfish (Ictalurus punctatus) liver. Stalling et al. (1973) found four metabolites after 2 hr incubation with liver microsomes. Three unidentified metabolites required NADPH for their production. These unidentified metabolites comprised 42% of the total metabolites present. The monoester of di-n-butylphthalate was the dominant metabolite accounting for 55% of the total metabolites and required no NADPH or oxygen for its production. Only 3% of the radioactivity in the microsomal preparation remained as parent compound.

In our metabolism study with fathead minnows exposed to 34.8 $\mu g \cdot L^{-1}$ of di-n-butylphthalate, 7 radioactive spots were separated by TLC from the homogenized whole fish supernatant after 1 day of exposure. After 72 hr of exposure, 8 spots were distinguishable by TLC. Only standards of parent compound and phthalic acid were co-chromatographed for possible identification of unknowns. Phthalic acid became detectable (Table 15) by the third day of exposure (1.9%) and remained nearly constant to the eleventh day (2.2%). The presence of parent di-n-butylphthalate on days 1 and 3 of exposure and a greatly reduced percentage on day 11 indicates that induced metabolism of di-n-butylphthalate may have occurred.

Protein binding of di-n-butylphthalate occurred in rainbow trout liver microsomes (Table 16) and to a more limited extent in Daphnia PMS. It was assumed that a portion of the radioactivity in the pellet (Table 15) from the fathead minnow metabolism study was protein bound di-n-butylphthalate.

Pentachlorophenol. LC_{50} values of 0.280 $mg \cdot L^{-1}$ at 96 hr and 46.0 $mg \cdot L^{-1}$ at 48 hr were obtained for Gammarus pseudolimnaeus and Tanytarsus dissimilis, respectively (Tables 6 and 7). Daphnia magna was the only other freshwater invertebrate species for which acute toxicity information was available where exposure concentrations were measured (U.S. EPA, 1980h). A 48 hr LC_{50} value of 0.6 $mg \cdot L^{-1}$ for Daphnia magna was reported (Adema, 1978). Static unmeasured 48 hr LC_{50} values for Daphnia magna have ranged from 0.24 to 0.80 $mg \cdot L^{-1}$, while Daphnia pulex and Daphnia cucullata had 48 hr LC_{50}s of 2.0 and 1.5 $mg \cdot L^{-1}$, respectively (Canton and Adema, 1978). Acute toxicities to 9 species of freshwater fish ranged from 0.034 to 0.600 $mg \cdot L^{-1}$ (U.S. EPA, 1980h).

Heptachlor. Exposure of Selenastrum capricornutum to heptachlor in two separate tests resulted in 96 hr EC_{50} values of 0.0381 and 0.0282 $mg \cdot L^{-1}$, based upon initial measured concentrations. Heptachlor concentrations declined rapidly, and the conversion product 1-hydroxychlordene was readily formed in considerable quantity (Tables 9 and 10). Values of 0.0394 and 0.0267 $mg \cdot L^{-1}$ were reported for our results in the heptachlor criteria document (U.S. EPA, 1980i), where EC_{50} values had been calculated by a different method. No other plant toxicity data have been published.

Eighteen freshwater species of animals, including fish and various invertebrates, had been used in static unmeasured acute tests with heptachlor (U.S. EPA, 1980i). No measured tests were reported. Toxicity values ranged from 0.0009 $mg \cdot L^{-1}$ for the stonefly (Pteronarcella badia) to 0.320 $mg \cdot L^{-1}$ for the goldfish. Selenastrum sensitivity to heptachlor on an acute basis appears to be similar to that for the bluegill sunfish, the scud (Gammarus lacustris), and Daphnia pulex.

Chlordane. A 48 hr LC_{50} estimate of 0.035 $mg \cdot L^{-1}$ to Daphnia magna was found in this study with technical chlordane. In a static unmeasured test, technical chlordane produced a 48 hr LC_{50} value of 0.097 $mg \cdot L^{-1}$ (Randall et al., 1979). A 96 hr EC_{50} value of 0.0284 $mg \cdot L^{-1}$ with Daphnia magna was observed in a measured test (Cardwell et al., 1977). Of 14 freshwater species of animals ranked for their sensitivities to chlordane, Daphnia magna was ranked number 11 (U.S. EPA, 1980j). However, almost all of the tests were static tests with unmeasured chlordane concentrations. Three fish tests had measured concentrations of chlordane, and in those tests the sensitivities of the fish species were quite similar to the sensitivity of Daphnia magna in this study. Flow-through measured tests with brook trout (Salvelinus fontinalis), fathead minnows, and bluegills gave 96 hr LC_{50} values of 0.047, 0.037, and 0.059 $mg \cdot L^{-1}$ of technical chlordane, respectively (Cardwell et al., 1977).

Toxaphene. The 96 hr EC_{50} value for Selenastrum capricornutum (50% reduction in dry weight as compared to controls) exposed to toxaphene was 0.38 $mg \cdot L^{-1}$, based upon initial measured toxaphene concentrations. Our results were incorrectly reported as 0.38 $\mu g \cdot L^{-1}$ in the toxaphene criteria document (U.S. EPA, 1980k). Toxaphene did not dissipate from the test solutions as rapidly as heptachlor (Tables 8, 9 and 10). No other toxicity tests with freshwater plants have been reported in the literature (U.S. EPA, 1980k). Studies with marine algae have shown that the productivity of natural phytoplankton communities is inhibited 90.8% with 1.0 $mg \cdot L^{-1}$ toxaphene present (Butler, 1963). In another study, death or complete inhibition of growth of marine algae occurred at much lower toxaphene concentrations ranging from 0.00015 to 0.150 $mg \cdot L^{-1}$ (Ukeles, 1962).

The acute toxicity of toxaphene to freshwater animals ranged from 0.0013 $mg \cdot L^{-1}$ for the stonefly, Claassenia sabulosa, to 0.180 $mg \cdot L^{-1}$ for the midge, Chironomus plumosus (U.S. EPA, 1980k). Comparison of our results using Selenastrum with animal species sensitivities indicates that this species of green algae is less sensitive than freshwater animals.

Arsenic^{+3}. In this study, 96 hr LC_{50} values of 14.2 and 14.4 $mg \cdot L^{-1}$ were obtained for the fathead minnow and flagfish, respectively (Tables 1 and 4). The 48 hr EC_{50} value for Daphnia magna was 1.54 $mg \cdot L^{-1}$ (Table 5), and the 96 hr LC_{50} value for Gammarus pseudolimnaeus was 0.875 $mg \cdot L^{-1}$ (Table 6).

From a review of the acute toxicity of trivalent inorganic arsenic to seven species of freshwater fish, LC_{50}s ranged from 13.3 to 41.8 $mg \cdot L^{-1}$ (U.S. EPA, 1980ℓ). These values were obtained from static unmeasured tests as well as flow-through measured tests (Clemens and Sneed, 1959; Cardwell et al., 1976; Inglis and Davis, 1972; Fish Pesticide Research Laboratory, 1980). Other Cladocean species have been tested for their acute sensitivities to arsenic^{+3}. Daphnia pulex had 48 hr EC_{50} values of 1.0 and 1.7 $mg \cdot L^{-1}$ (Sanders and Cope, 1966; Fish Pesticide Research Laboratory, 1980), and Simocephalus serrulatus had a 48 hr EC_{50} of 0.812 $mg \cdot L^{-1}$ (Sanders and Cope, 1966).

Our acute value for Gammarus pseudolimnaeus is reported as 0.879 $mg \cdot L^{-1}$ in the arsenic criteria document (U.S. EPA, 1980ℓ). This value was a mean of two replicates, whereas 0.875 $mg \cdot L^{-1}$ is a single value for the two replicates pooled. Exposure of Gammarus pseudolimnaeus to an arsenic^{+3} concentration of 0.961 $mg \cdot L^{-1}$ for 7 days resulted in 80% mortality (Spehar et al., 1980). Of 12 species of fish and invertebrates combined, Gammarus pseudolimnaeus was the second most sensitive species in acute tests (U.S. EPA, 1980ℓ).

In the chronic study with Daphnia magna, significant reductions ($p<0.01$) in the number of young produced and length of adults were observed at mean arsenic^{+3} concentrations of 1.32 $mg \cdot L^{-1}$ and above (Table 11). The "no-effect" range was 0.633 to 1.32 $mg \cdot L^{-1}$. Thus, the concentration that caused significant adverse chronic effects was not much different from the 48 hr EC_{50} of 1.54 $mg \cdot L^{-1}$. No other chronic studies with invertebrates have been reported

(U.S. EPA, 1980ℓ).

In the early life-stage test with fathead minnows and arsenic^{+3}, wet weight and length of the juvenile fish at 24 days post-hatch were significantly reduced ($p<0.01$) at concentrations of 4.30 mg·L^{-1} and above (Table 12). The "no-effect" range was from 2.13 to 4.30 mg·L^{-1}, or from 15 to 30 percent of the 96 hr LC$_{50}$ concentration of 14.2 mg·L^{-1}.

In the first early life-stage test using flagfish, the exposure concentrations were too low to cause any effects (Table 14). When this test was repeated (Table 15), a reduction ($p<0.05$) in length of juvenile fish at 25 days post-hatch was observed at 4.12 mg·L^{-1}. At the two higher exposures length and weight were both reduced at the 99% probability level. The "no-effect" range was 2.13 to 4.12 mg·L^{-1}, or from 15 to 29 percent of the 96 hr LC$_{50}$ concentration of 14.4 mg·L^{-1}. No chronic toxicity tests with fish have been reported for either freshwater or marine species (U.S. EPA, 1980ℓ).

Chromium^{+6}. LC$_{50}$ values (96 hr) of 0.0671 and 0.0941 mg·L^{-1} were determined for Gammarus pseudolimnaeus with flow-through measured and static unmeasured tests, respectively (Table 6). A 48 hr LC$_{50}$ value of 57.3 mg·L^{-1} was determined for the midge, Tanytarsus dissimilis (Table 7).

From a review on hexavalent chromium (U.S. EPA, 1980m), the acute toxicity to freshwater animals is highly variable with species and extends over four orders of magnitude. The lowest LC$_{50}$ value reported was 0.031 mg·L^{-1} for Daphnia magna at 72 hr (Debelak, 1975). The highest LC$_{50}$ value reported was 249 mg·L^{-1} for goldfish at 96 hr (Dowden and Bennett, 1965). Invertebrate species were generally more sensitive to chromium than fish species (U.S. EPA, 1980m). However, Tanytarsus with a 48 hr LC$_{50}$ value of 57.3 mg·L^{-1} (rather than 59.9 mg·L^{-1} as listed in the criteria document), was the most tolerant freshwater invertebrate with acute sensitivity similar to that for brook and rainbow trout (Benoit, 1976).

Lead^{+2}. A 96 hr LC_{50} value of 0.140 $mg \cdot L^{-1}$ was determined for Gammarus pseudolimnaeus (Table 6) and a 48 hr LC_{50} value of 224 $mg \cdot L^{-1}$ was obtained for Tanytarsus dissimilis (Table 7). A flow-through measured test with the same scud species was conducted by Spehar et al. (1978). They obtained a 96 hr LC_{50} value of 0.124 $mg \cdot L^{-1}$, and a 28 day LC_{50} value of 0.028 $mg \cdot L^{-1}$. Of the freshwater animals tested, Gammarus was the most sensitive species to lead in acute tests (U.S. EPA, 1980n).

The toxicity of lead decreases with increased water hardness (U.S. EPA, 1980n). For test water with hardness below 150 $mg \cdot L^{-1}$ as $CaCO_3$, Tanytarsus dissimilis was the most tolerant of freshwater animals (U.S. EPA, 1980n).

Mercury^{+2}. A 96 hr LC_{50} value of 150 $\mu g \cdot L^{-1}$ was obtained with the fathead minnow (Table 1). The "no-effect" concentration from an early life-stage study with this species was less than the lowest exposure concentration of 0.23 $\mu g \cdot L^{-1}$ (Table 13).

A 96 hr LC_{50} value of 168 $\mu g \cdot L^{-1}$ was found for fathead minnows of approximately 3 months in age (Snarski and Olson, 1982). The fish in our study were approximately 1 month old. Mercuric chloride 96 hr LC_{50} values for rainbow trout fingerlings were 400, 280, and 200 $\mu g \cdot L^{-1}$ at temperatures of 5, 10, and 20 C (Macleod and Pessah, 1973). In other tests with $HgCl_2$ and rainbow trout, a 96 hr LC_{50} value of 210 $\mu g \cdot L^{-1}$ was obtained at 16.7 C (Matida et al., 1971) and a 24 hr LC_{50} value of 903 $\mu g \cdot L^{-1}$ at 10 C (Wobeser, 1975). LC_{50} values for various freshwater invertebrate species exposed to $HgCl_2$ ranged from 5 $\mu g \cdot L^{-1}$ for Daphnia magna (Biesinger and Christensen, 1972) to 2.0 $mg \cdot L^{-1}$ for a stonefly (Acroneuria lycorius), a mayfly (Ephemerella subvaria), and a caddisfly (Hydropsyche betteni) (Warnick and Bell, 1969).

In a full life-cycle chronic exposure of fathead minnows to $HgCl_2$, no spawning occurred at or above 1.02 $\mu g \cdot L^{-1}$, and total egg production was only 46 and 54% of controls at mean exposure concentrations of 0.26 and 0.50 $\mu g \cdot L^{-1}$, respectively (Snarski and Olson, 1982). Progeny after 30 days of exposure were shorter and lighter than controls at all exposure concentrations. The lowest mean exposure level was 0.26 $\mu g \cdot L^{-1}$. Therefore, the "no-effect" range based on growth parameters of offspring was below the lowest exposure of 0.26 $\mu g \cdot L^{-1}$. Rainbow trout exposed to $HgCl_2$ demonstrated growth retardation at concentrations of 21 $\mu g \cdot L^{-1}$ or greater (Matida et al., 1971). A permissible concentration range based on growth was reported at between 2.1 and 21 $\mu g \cdot L^{-1}$.

Embryonic or larval forms of aquatic animals have generally been more sensitive to mercury than adults (Taylor, 1979). $HgCl_2$ was toxic to embryos and teratogenic in marine killifish (Fundulus heteroclitus) at 10 $\mu g \cdot L^{-1}$ (Weis and Weis, 1977) and in Japanese medaka (Oryzias latipes) at 15 $\mu g \cdot L^{-1}$ (Heisinger and Green, 1975). Scoliosis (lateral spinal curvature) occurred in 18 and 38% of the smaller undeveloped fathead minnows exposed to 2.01 and 3.69 $\mu g \cdot L^{-1}$ $HgCl_2$, respectively, for 41 weeks (Snarski and Olson, 1982). Significant teratogenic effects were observed in the present study at concentrations of 1.8 and 3.7 $\mu g \cdot L^{-1}$.

The "no-effect" ranges for Daphnia magna exposed to $HgCl_2$ in flow-through and renewed static tests were 0.7 to 1.3 $\mu g \cdot L^{-1}$ and 0.9 - 1.8 $\mu g \cdot L^{-1}$, respectively (Biesinger et al., 1982). No other chronic studies with $HgCl_2$ and freshwater organisms have been published (U.S. EPA, 1980 o).

Nickel^{+2}. A 48 hr LC_{50} value of 0.915 $mg \cdot L^{-1}$ with Daphnia magna was determined (Table 5). This was reported as 0.865 $mg \cdot L^{-1}$ in the criteria document when determined by a different method (U.S. EPA, 1980p). The acute toxicity of nickel decreases with increased water hardness (U.S. EPA, 1980p). Acute toxicity values for freshwater invertebrates from studies with hardness similar to ours (40 - 50 $mg \cdot L^{-1}$) have ranged from 0.510 $mg \cdot L^{-1}$ in Daphnia magna (Biesinger and Christensen, 1972) to 33.5 $mg \cdot L^{-1}$ in a stonefly, Acroneuria lycorias (Warnick and Bell, 1969).

Silver^{+1}. LC_{50} values (96 hr) of 0.0107 $mg \cdot L^{-1}$, 0.0092 $mg \cdot L^{-1}$, and 0.00449 $mg \cdot L^{-1}$ were determined for the fathead minnow, flagfish, and scud, respectively (Tables 1, 4 and 6). The 48 hr LC_{50} for Tanytarsus dissimilis was 3.17 $mg \cdot L^{-1}$ (Table 7). In the silver criteria document (U.S. EPA, 1980q), our LC_{50} values are reported as 0.011 $mg \cdot L^{-1}$ and 0.0096 $mg \cdot L^{-1}$ for fathead minnows and flagfish, respectively. The 48 hr LC_{50} for Tanytarsus dissimilis was listed as 3.2 $mg \cdot L^{-1}$. Deviations in values reported here from those published in the criteria document are due to rounding or the use of a different approach in calculating LC_{50} values. However, the scud LC_{50} value is incorrectly listed as 4.5 $mg \cdot L^{-1}$. It should be 4.5 $\mu g \cdot L^{-1}$.

The acute toxicity of silver decreases as water hardness increases (U.S. EPA, 1980q). From a review of the acute toxicities of silver to other freshwater invertebrates (U.S. EPA, 1980q), LC_{50} values ranged from 0.25 $\mu g \cdot L^{-1}$ for Daphnia magna in an unpublished study by Chapman and co-workers from the EPA laboratory in Corvallis, Oregon, to 1.4 $mg \cdot L^{-1}$ for the rotifer, Philodena acuticornis (Buikema et al., 1974). The midge LC_{50} value of 3.17 $mg \cdot L^{-1}$ represents the least sensitive acute response.

Most of the acute toxicity studies with freshwater fish have been conducted with rainbow trout and fathead minnows (U.S. EPA, 1980q). In water of similar hardness as in our study, rainbow trout had LC_{50} values ranging from

0.0069 to 0.110 mg·L^{-1} in flow-through measured tests (Lemke, 1981). In softer water (20 - 31 mg·L^{-1} as $CaCO_3$), rainbow trout LC_{50} values ranged from 0.0053 to 0.0081 mg·L in three tests (Davies _et_ _al_., 1978). Fathead minnow LC_{50} values ranged from 0.0039 to 0.030 mg·L^{-1} in water with a hardness range of 46 - 54 mg·L^{-1} as $CaCO_3$ (Lemke, 1981).

Selenium^{+4}. The 48 hr LC_{50} value was 42.5 mg·L^{-1} for _Tanytarsus_ _dissimilis_ (Table 7). Our test result was reported as 42.4 mg·L^{-1} in the selenium criteria document (U.S. EPA, 1980r). The midge was the most tolerant of freshwater invertebrates from acute tests (U.S. EPA, 1980r). LC_{50} values for other invertebrate species range from 0.340 mg·L^{-1} for the scud, _Hyallela_ _azteca_ (Halter _et_ _al_., 1980) to 24.1 mg·L^{-1} in a snail, _Physa_ sp. (Reading, 1979).

Cyanide. The 48 hr LC_{50} value for _Tanytarsus_ _dissimilis_ was 2.36 mg·L^{-1} for free cyanide expressed as HCN and 2.49 mg·L^{-1} when expressed as CN^- (Table 7). The criteria document for cyanides (U.S. EPA, 1980s) has reported our test result as 2.24 mg·L^{-1}. Upon recalculation, we have arrived at a final 48 hr LC_{50} of 2.49 mg·L^{-1}.

Four other invertebrate species had been used in acute toxicity tests with cyanide (U.S. EPA 1980s). LC_{50} values were 0.431 mg·L^{-1} for the snail, _Physa_ _heterostropha_, 0.083 mg·L^{-1} for _Daphnia_ _pulex_, 0.167 mg·L^{-1} for the scud, _Gammarus_ _pseudolimnaeus_, and 2.326 mg·L^{-1} for the isopod, _Asellus_ _communis_ (Cairns and Scheier, 1958; Patrick _et_ _al_., 1968; Lee, 1976; Oseid and Smith, 1979). Of 15 species of freshwater fauna tested for acute sensitivity to cyanide, _Tanytarsus_ _dissimilis_ was the most tolerant (U.S. EPA, 1980s).

Microsomal Metabolism and Binding of Chlorinated Hydrocarbons

It is apparent that rainbow trout and _Daphnia_ both possess an active mixed function oxidase system which may play an important role in detoxication of chlorinated hydrocarbons. Perhaps the initial oxidation of these compounds occurs via the mixed function oxidase system. Toxicity may be related to irreversible protein binding, and lipid peroxidation causing disruption of the endoplasmic membrane. Further metabolic studies of these chemicals should be conducted to determine their interaction with cellular components, and to identify specific metabolites.

Mixed Function Oxidase Activity

Our data indicate (Table 23) that aquatic organisms have measurable but lower mixed function oxidase activity than mammals. However, with similar metabolic systems, the mechanisms leading to toxicity and neoplasia are presumed to be qualitatively similar in all organisms. Therefore, studies with aquatic organisms can be used for important functions. The first is for laboratory screening. Because they are relatively easy to rear, they are economically attractive test organisms. The second is for environmental monitoring. Aquatic organisms are currently being used as sentinels to signal environmental contamination (Black _et al_., 1980). In summary, both laboratory and field studies using aquatic organisms are recommended for programs in comparative pharmacological testing, short-term screening and environmental monitoring.

TABLE 23. COMPARISON OF MIXED FUNCTION OXIDASE MEASUREMENTS BETWEEN MAMMALS AND SEVERAL NON-MAMMALIAN AQUATIC ORGANISMS

Enzymes[a]	Human	Male Rat[c]	Rainbow Trout[d]	Daphnia[d]	Blue Crab[e]
Cytochrome P-450	0.60 ± 0.10[b]	0.72 ± 0.08	0.28 ± 0.10	ND[f]	0.18 ± 0.08
Cytochrome b_5	0.49 ± 0.06[b]	0.30 ± 0.08	0.19 ± 0.05	ND	-
NADPH Cytochrome c reductase	102.6 ± 14.6[b]	96 ± 20	15.9 ± 2.2	42.0 ± 5.3	5.2 ± 4.8
Aniline hydroxylase	8.7 ± 6.8[c]	22 ± 5	0.55 ± 0.01	-	0.016 ± 0.008

[a] Activities expressed as in Table 22.

[b] Ahmad and Black, 1977.

[c] Kato, 1979.

[d] This study.

[e] James *et al.*, 1979.

[f] Not detectable.

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APPENDIX A

SUMMARIES OF CONDITIONS AND WATER CHARACTERISTICS FOR TOXICITY TESTS

TABLE A-1. SUMMARY OF TEST CONDITIONS AND TEST WATER CHARACTERISTICS FOR MEASURED TOXICITY TESTS WITH FISH, SELECTED INVERTEBRATES AND ALGAE

Test Compound	Test Organism	Test Duration	Water Supply	Temperature °C $\bar{x}$ ± s.d. (range)	% Dissolved Oxygen $\bar{x}$ ± s.d. (range)	Hardness[a] $\bar{x}$ ± s.d.	Alkalinity[a] $\bar{x}$ ± s.d.	Acidity[a] $\bar{x}$ ± s.d.	pH $\bar{x}$ ± s.d.
Hexachloroethane	*Tanytarsus dissimilis*	48 hr	L. Superior	~20	93.4 ± 3.2 (88.7 - 98.7) n=36	46.7 ± 0.9 n=5	44.0 ± 1.9 n=6	2.9 ± 0.7 n=5	7.6 ± 0.0 n=6
Hexachloroethane	*Salmo gairdneri*	192 hr	L. Superior	11.6 ± 0.4 (10.9 - 12.7) n=80	69.2 ± 6.5 (59.0 - 77.1) n=13	42.5 ± 1.0 n=4	46.6 ± 0.5 n=4	2.4 ± 0.1 n=4	7.2 ± 0.0 n=4
Tetrachloroethylene	*Salmo gairdneri*	96 hr	L. Superior	11.6 ± 0.2 (11.2 - 12.0) n=40	81.6 ± 5.2 (73.9 - 92.4) n=11	44.1 ± 0.5 n=4	46.9 ± 0.6 n=4	2.1 ± 0.1 n=4	7.1 ± 0.0 n=4
Tetrachloroethylene/DMF	*Salmo gairdneri*	96 hr	L. Superior	12.2 ± 0.3 (11.5 - 12.5) n=44	80.7 ± 5.4 (71.2 - 86.2) n=10	46.2 ± 0.8 n=3	46.5 ± 0.5 n=3	2.0 ± 0.1 n=3	7.2 ± 0.0 n=3
Tetrachloroethylene	*Tanytarsus dissimilis*	48 hr	L. Superior	~ 20	97.0 ± 1.2 (95.3 - 99.8) n=22	46.4 ± 1.0 n=5	43.0 ± 1.0 n=5	2.8 ± 0.3 n=5	7.5 ± 0.0 n=5
1,2-Dichlorobenzene	*Salmo gairdneri*	144 hr	L. Superior	12.0 ± 0.2 (11.6 - 12.4) n=60	82.4 ± 7.4 (73.8 - 99.4) n=13	47.3 ± 0.1 n=7	47.7 ± 1.1 n=7	2.0 ± 0.1 n=7	7.5 ± 0.1 n=7
1,2-Dichlorobenzene	*Tanytarsus dissimilis*	48 hr	L. Superior	~ 20	87.2 ± 3.3 (80.9 - 90.9) n=12	47.0 ± 2.0 n=6	46.0 ± 3.0 n=6	2.3 ± 0.3 n=6	7.6 ± 0.0 n=6
1,4-Dichlorobenzene	*Salmo gairdneri*	96 hr	L. Superior	11.9 ± 0.3 (11.2 - 12.7) n=57	84.0 ± 7.6 (73.7 - 100.3) n=10	45.3 ± 0.8 n=7	44.6 ± 1.7 n=7	4.1 ± 1.0 n=7	6.8 ± 0.1 n=7
1,4-Dichlorobenzene	*Tanytarsus dissimilis*	48 hr	L. Superior	~ 20	97.5 ± 2.1 (95.3 - 99.8) n=22	45.9 ± 1.6 n=5	44.8 ± 2.4 n=5	2.7 ± 0.5 n=5	7.6 ± 0.0 n=6
1,2,4-Trichlorobenzene	*Salmo gairdneri*	192 hr	L. Superior	12.6 ± 0.4 (11.4 - 13.4) n=96	86.4 ± 7.6 (66.5 - 95.3) n=16	47.7 ± 1.3 n=9	53.1 ± 10.1 n=9	2.6 ± 0.7 n=8	7.4 ± 0.1 n=9

TABLE A-1 Cont. SUMMARY OF TEST CONDITIONS AND TEST WATER CHARACTERISTICS FOR MEASURED TOXICITY TESTS WITH FISH, SELECTED INVERTEBRATES AND ALGAE

Test Compound	Test Organism	Test Duration	Water Supply	Temperature °C $\bar{x}$ ± s.d. (range)	% Dissolved Oxygen $\bar{x}$ ± s.d. (range)	Hardness[a/] $\bar{x}$ ± s.d.	Alkalinity[a/] $\bar{x}$ ± s.d.	Acidity[a/] $\bar{x}$ ± s.d.	pH $\bar{x}$ ± s.d.
Pentachlorobenzene	*Salmo gairdneri*	144 hr	L. Superior	12.7 ± 0.8 (12.2 - 13.5) n=79	83.4 ± 7.8 (67.7 - 96.0) n=14	46.2 ± 1.8 n=4	45.4 ± 1.9 n=4	3.6 ± 0.4 n=4	7.4 ± 0.0 n=4
Hexachlorobenzene	*Salmo gairdneri*	96 hr	L. Superior	11.2 ± 0.8 (10.5 - 12.8) n=30	n.d.	44.3 ± 2.1 n=3	49.0 ± 1.0 n=3	0.3 ± 0.1 n=3	7.5 ± 0.4 n=3
Hexachlorobenzene	*Lepomis macrochirus*	96 hr	L. Superior	23.3 ± 0.5 (21.1 - 23.8) n=36	89.9 ± 11.3 (72.8 - 100) n=15	45.4 ± 1.5 n=3	42.5 ± 0.7 n=3	1.9 ± 1.1 n=3	7.7 ± 0.1 n=3
Hexachlorobenzene	*Tanytarsus dissimilis*	48 hr	L. Superior	23.9 ± 1.2 (22.5 - 25.6) n=8	85.9 ± 0.0 (85.9 - 85.9) n=4	40.7 ± 2.8 n=4	43.2 ± 1.3 n=4	1.5 ± 0.6 n=4	7.8 ± 0.0 n=4
Hexachlorobutadiene	*Salmo gairdneri*	168 hr	L. Superior	11.9 ± 0.4 (10.9 - 13.0) n=87	81.2 ± 0.7 (80.4 - 82.1) n=7	45.9 ± 0.6 n=7	45.7 ± 0.8 n=7	2.0 ± 0.1 n=7	7.5 ± 0.0 n=7
Hexachlorobutadiene	*Lepomis macrochirus*	192 hr	L. Superior	25.2 ± 0.2 (24.7 - 25.8) n=102	93.0 ± 3.1 (89.2 - 99.9) n=17	41.7 ± 2.7 n=12	46.5 ± 6.0 n=12	2.2 ± 0.5 n=12	7.6 ± 0.2 n=12
Pentachlorophenol	*Gammarus pseudolimnaeus*	96 hr	L. Superior	17.1 ± 0.5 (16.3 - 18.0) n=56	95.2 ± 3.7 (84.9 - 98.9) n=11	47.5 ± 2.2 n=4	62.8 ± 8.1 n=4	4.8 ± 2.0 n=4	7.2 ± 0.2 n=4
Pentachlorophenol	*Tanytarsus dissimilis*	48 hr	L. Superior	23.8 ± 0.3 (23.5 - 24.0) n=3	79.0 ± 4.6 (68.7 - 84.3) n=12	44.5[b/] n=1	55.0[b/] n=1	0.5[b/] n=1	7.9 ± 0.2[c/] n=12
Heptachlor	*Selenastrum capricornutum*	96 hr	reconstituted deionized	~ 24	n.d.	n.d.	n.d.	n.d.	n.d.
Toxaphene	*Selenastrum capricornutum*	96 hr	reconstituted deionized	~ 24	n.d.	n.d.	n.d.	n.d.	n.d.

TABLE A-1 Cont. SUMMARY OF TEST CONDITIONS AND TEST WATER CHARACTERISTICS FOR MEASURED TOXICITY TESTS WITH FISH, SELECTED INVERTEBRATES AND ALGAE

Test Compound	Test Organism	Test Duration	Water Supply	Temperature °C $\bar{x}$ ± s.d. (range)	% Dissolved Oxygen $\bar{x}$ ± s.d. (range)	Hardness$^{a/}$ $\bar{x}$ ± s.d.	Alkalinity$^{a/}$ $\bar{x}$ ± s.d.	Acidity$^{a/}$ $\bar{x}$ ± s.d.	pH $\bar{x}$ ± s.d.
Arsenic^{+3}	*Pimephales promelas*	96 hr	City of Superior	24.2 ± 0.6 (23.0 - 25.3) n=49	83.8 ± 2.5 (77.8 - 88.8) n=9	49.9 ± 0.7 n=5	37.2 ± 0.8 n=5	3.4 ± 0.1 n=5	7.2 ± 0.1 n=8
Arsenic^{+3}	*Pimephales promelas*	31 days	City of Superior	23.0 ± 2.7 (13.5 - 26.7) n=360	79.6 ± 7.4 (63.7 - 93.3) n=108	49.2 ± 1.4 n=8	38.0 ± 2.2 n=8	3.3 ± 0.3 n=8	7.2 ± 0.1 n=20
Arsenic^{+3}	*Jordanella floridae*	96 hr	City of Superior	25.8 ± 0.7 (24.1 - 27.2) n=50	86.9 ± 2.5 (82.0 - 89.2) n=9	49.9 ± 0.7 n=5	37.2 ± 0.8 n=5	3.4 ± 0.1 n=5	7.2 ± 0.1 n=8
Arsenic^{+3} (test 1)	*Jordanella floridae*	38 days	L. Superior	24.8 ± 1.3 (21.8 - 27.5) n=168	83.6 ± 5.9 (70.2 - 92.5) n=96	47.0 ± 0.0 n=4	n.d.	4.2 ± 0.3 n=4	7.4 ± 0.0 n=4
Arsenic^{+3} (test 2)	*Jordanella floridae*	31 days	City of Superior	24.4 ± 2.8 (13.4 - 28.6) n=360	85.8 ± 4.7 (73.1 - 95.8) n=108	49.1 ± 1.4 n=8	38.1 ± 2.1 n=8	3.3 ± 0.2 n=8	7.2 ± 0.1 n=20
Arsenic^{+3}	*Gammarus pseudo-limnaeus*	96 hr	L. Superior	18.4 ± 0.9 (17.3 - 19.5) n=54	99.3 ± 1.3 (97.3 - 100.2) n=6	46.3 ± 0.5 n=4	43.4 ± 1.5 n=4	3.0 ± 0.0 n=4	7.7 ± 0.2 n=4
Chromium^{+6}	*Gammarus pseudo-limnaeus*	96 hr	L. Superior	17.4 ± 0.5 (16.5 - 18.0) n=54	96.7 ± 0.7 (95.1 - 97.4) n=10	47.8 ± 0.6 n=4	53.8 ± 1.0 n=4	6.9 ± 1.2 n=4	7.6 ± 0.1 n=4
Chromium^{+6}	*Tanytarsus dissimilis*	48 hr	L. Superior	20.4 ± 0.1 (20.3 - 20.5) n=3	86.6 ± 1.4 (84.3 - 89.8) n=10	47.0$^{b/}$ n=1	187.5$^{b/}$ n=1	2.5$^{b/}$ n=1	7.5 ± 0.2 n=4
Lead^{+2}	*Gammarus pseudo-limnaeus*	96 hr	L. Superior	17.6 ± 0.4 (17.0 - 18.5) n=52	94.7 ± 2.1 (91.7 - 96.8) n=11	48.3 ± 0.9 n=4	40.8 ± 4.3 n=4	1.4 ± 0.2 n=4	6.5 ± 0.2 n=4
Lead^{+2}	*Tanytarsus dissimilis*	48 hr	L. Superior	18.6 ± 0.6 (17.6 - 19.5) n=36	78.9 ± 4.2 (70.0 - 83.9) n=10	n.d.	12.1$^{b/}$ n=1	65.0$^{b/}$ n=1	4.9 - 6.9$^{d/}$ n=12

TABLE A-1 Cont. SUMMARY OF TEST CONDITIONS AND TEST WATER CHARACTERISTICS FOR MEASURED TOXICITY TESTS WITH FISH, SELECTED INVERTEBRATES AND ALGAE

Test Compound	Test Organism	Test Duration	Water Supply	Temperature °C $\bar{x} \pm$ s.d. (range)	% Dissolved Oxygen $\bar{x} \pm$ s.d. (range)	Hardness[a] $\bar{x} \pm$ s.d.	Alkalinity[a] $\bar{x} \pm$ s.d.	Acidity[a] $\bar{x} \pm$ s.d.	pH $\bar{x} \pm$ s.d.
Mercury^{+2}	Pimephales promelas	96 hr	L. Superior	25.7 ± 0.6 (24.7 - 27.3) n=51	93.0 ± 3.6 (88.5 - 97.9) n=10	42.8 ± 0.0 n=3	41.2 ± 0.2 n=3	2.4 ± 0.0 n=3	7.6 ± 0.0 n=3
Mercury^{+2}	Pimephales promelas	35 days	L. Superior	25.0 ± 0.6 (23.7 - 26.3) n=132	81.0 ± 9.6 (65.1 - 91.2) n=17	45.6 ± 3.2 n=2	41.3 ± 0.2 n=2	2.6 ± 0.3 n=2	7.5 ± 0.1 n=2
Silver^{+1}	Pimephales promelas	96 hr	City of Superior	23.4 ± 2.6 (18.5 - 26.1) n=36	85.5 ± 3.5 (79.4 - 89.4) n=8	46.0 ± 1.4 n=2	36.2 ± 3.9 n=2	3.5 ± 0.1 n=2	7.2 ± 0.1 n=7
Silver^{+1}	Jordanella floridae	96 hr	L. Superior	24.7 ± 0.5 (23.9 - 25.8) n=51	92.4 ± 5.9 (82.7 - 98.8) n=10	44.5 ± 4.0 n=4	43.5 ± 1.7 n=4	2.5 ± 0.0 n=4	7.8 ± 0.0 n=4
Silver^{+1}	Gammarus pseudo-limnaeus	96 hr	L. Superior	20.0 ± 0.5 (19.1 - 20.7) n=44	95.0 ± 2.6 (91.2 - 98.5) n=10	48.1 ± 1.4 n=4	45.2 ± 1.3 n=4	1.1 ± 0.2 n=4	7.5 ± 0.2 n=4
Silver^{+1}	Tanytarsus dissimilis	48 hr	L. Superior	19.8 ± 0.3 (19.5 - 20.0) n=3	94.5 ± 4.0 (88.7 - 97.6) n=4	47.9[a] n=1	42.0[b] n=1	1.5[b] n=1	7.6 ± 0.1 n=12
Selenium^{+4}	Tanytarsus dissimilis	48 hr	L. Superior	19.0 ± 0.8 (18.1 - 19.8) n=4	97.4 ± 2.3 (93.9 - 100.3) n=10	48.0[b] n=1	n.d.	44.0[b] n=1	3.2 - 7.6[d] n=10
Cyanide	Tanytarsus dissimilis	48 hr	L. Superior	~ 20	99.0 ± 1.2 (97.0 - 99.8) n=5	46.8 ± 1.6 n=6	46 - 82[c] n=6	0 - 2.0[d] n=6	7.6 - 9.2[c] n=6

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[a] Values expressed as mg·L^{-1} of $CaCO_3$.

[b] Analysis performed on single pooled sample from selected exposure concentrations.

[c] Values increased with increased toxicant concentration.

[d] Values decreased with increased toxicant concentration.

TABLE A-2. SUMMARY OF CONDITIONS AND WATER CHARACTERISTICS FOR MEASURED TOXICITY TESTS WITH *DAPHNIA MAGNA* IN LAKE SUPERIOR WATER

Compound	Test Duration	Temperature °C $\bar{x}$ ± s.d. (Range)	% Dissolved Oxygen in New Solutions $\bar{x}$ ± s.d. (Range) Fed or Unfed	% Dissolved Oxygen in Old Solutions $\bar{x}$ ± s.d. (Range)	
				Fed	Unfed
1,2-Dichloroethane	48 hr	20 ± 1	n.d.	n.d.	n.d.
1,2-Dichloroethane	28 day	20 ± 1	97.3 ± 3.4 (89.8 - 102.2) n=32	73.8 ± 9.6 (55.5 - 96.3) n=57	-
1,1,2-Trichloroethane	48 hr	20 ± 1	90.1 ± 1.4 (88.9 - 91.8) n=3	74.2 ± 4.9 (67.2 - 78.5) n=4	109.1 ± 4.3 (104.1 - 114.2) n=4
1,1,2-Trichloroethane	28 day	20 ± 1	94.1 ± 6.0 (79.5 - 102.5) n=30	69.1 ± 10.2 (39.8 - 86.2) n=63	-
1,1,2,2-Tetrachloroethane	48 hr	20 ± 1	91.2 ± 0.9 (90.2 - 92.2) n=4	74.7 ± 4.3 (69.4 - 77.4) n=3	92.6 ± 1.3 (91.8 - 94.1) n=3
1,1,2,2-Tetrachloroethane (test 1)	28 day	20 ± 1	n.d.	33.5 ± 18.8 (5.5 - 72.1) n=136	-
1,1,2,2-Tetrachloroethane (test 2)	28 day	20 ± 1	92.4 ± 4.4 (87.7 - 97.2) n=4	64.4 ± 16.8 (22.6 - 79.2) n=29	
Pentachloroethane	48 hr	20 ± 1	93.4 ± 1.7 (92.0 - 95.4) n=3	82.2 ± 4.9 (75.0 - 86.1) n=4	94.8 ± 1.4 (93.0 - 96.3) n=4
Hexachloroethane	48 hr	20 ± 1	87.1 ± 0.8 (86.1 - 87.9) n=4	69.0 ± 7.0 (59.0 - 74.5) n=4	87.5 ± 1.6 (80.2 - 89.4) n=4

TABLE A-2 Cont. SUMMARY OF CONDITIONS AND WATER CHARACTERISTICS FOR MEASURED TOXICITY TESTS WITH DAPHNIA MAGNA IN LAKE SUPERIOR WATER

Compound	Test Duration	Temperature °C $\bar{x}$ ± s.d. (Range)	% Dissolved Oxygen in New Solutions $\bar{x}$ ± s.d. (Range) Fed or Unfed	% Dissolved Oxygen in Old Solutions $\bar{x}$ ± s.d. (Range)	
				Fed	Unfed
Tetrachloroethylene	48 hr	20 ± 1	n.d.	45.2 ± 7.1 (37.5 - 53.3) n=4	98.6 ± 6.2 (89.4 - 102.5) n=4
Tetrachloroethylene	28 day	20 ± 1	98.1 ± 8.6 (80.4 - 114.7) n=39	59.6 ± 13.7 (26.2 - 74.8) n=50	-
1,3-Dichlorobenzene	48 hr	20 ± 1	n.d.	83.7 ± 1.8 (82.5 - 85.7) n=3	94.9 ± 0.6 (94.4 - 95.4) n=3
1,3-Dichlorobenzene	28 day	20 ± 1	92.6 ± 4.4 (84.4 - 99.3) n=27	71.2 ± 9.2 (52.4 - 83.4) n=25	-
1,2,4-Trichlorobenzene	48 hr	20 ± 1	n.d.	n.d.	n.d.
1,2,4-Trichlorobenzene	28 day	20 ± 1	90.6 ± 3.4 (81.5 - 94.7) n=19	72.0 ± 9.5 (48.8 - 91.8)	
Di-n-butylphthalate	48 hr	20.9 ± 0.1 (20.8 - 21.0) n=3	n.d.	-	93.3 ± 1.5 (90.5 - 95.0) n=9
Chlordane	48 hr	~21	n.d.	-	88.2[b] n=1
Arsenic^{+3}	28 day	21.5 ± 3.0 (15 - 26) n=11	n.d.	86.9 ± 9.9 (70.1 - 101.8) n=71	

TABLE A-2 Cont. SUMMARY OF CONDITIONS AND WATER CHARACTERISTICS FOR MEASURED TOXICITY TESTS WITH *DAPHNIA MAGNA* IN LAKE SUPERIOR WATER

Compound	Test Duration	Temperature °C $\bar{x}$ ± s.d. (Range)	% Dissolved Oxygen in New Solutions $\bar{x}$ ± s.d. (Range) Fed or Unfed	% Dissolved Oxygen in Old Solutions $\bar{x}$ ± s.d. (Range)	
				Fed	Unfed
Arsenic^{+3} [c]	48 hr[c]	14.8 ± 0.8 (13.4 - 16.0) n=140	91.6 ± 0.0 (91.6 - 91.6) n=8	90.4 ± 0.0 (90.4 - 90.4) n=4	91.0 ± 0.3 (90.7 - 91.4) n=4
Nickel^{+2}	48 hr	~20	n.d.	-	87.7 ± 1.6 (85.8 - 89.0 n=4

TABLE A-2 Cont. SUMMARY OF CONDITIONS AND WATER CHARACTERISTICS FOR MEASURED TOXICITY TESTS WITH DAPHNIA MAGNA IN LAKE SUPERIOR WATER

Compound	Test Duration	Hardness[a]	Alkalinity[a] $\bar{x}$ ± s.d.	Acidity[a] $\bar{x}$ ± s.d.	pH of New Solution $\bar{x}$ ± s.d. Fed or Unfed	pH of Old Solution $\bar{x}$ ± s.d. Fed	Unfed
1,2-Dichloroethane	48 hr	44.5	37.0	n.d.	n.d.	n.d.	n.d.
1,2-Dichloroethane	28 day	44.0 ± 0.0 n=2	41.5 ± 0.7 n=2	n.d.	7.5 ± 0.1 n=33	7.1 ± 0.1 n=32	-
1,1,2-Trichloroethane	48 hr	43.0	37.0	n.d.	n.d.	7.4 ± 0.1 n=4	7.7 ± 0.1 n=4
1,1,2-Trichloroethane	28 day	44.4 ± 0.9 n=4	39.9 ± 3.5 n=4	n.d.	7.6 ± 0.1 n=27	7.0 ± 0.1 n=59	-
1,1,2,2-Tetrachloroethane	48 hr	45.0	42.0	n.d.	7.1 ± 0.2 n=4	7.0 ± 0.1 n=3	7.3 ± 0.3 n=3
1,1,2,2-Tetrachloroethane (test 1)	28 day	44.1 ± 0.9 n=4	41.6 ± 1.2 n=4	n.d.	n.d.	n.d.	-
1,1,2,2-Tetrachloroethane (test 2)	28 day	45.3 ± 0.3 n=3	43.3 ± 1.6 n=3	n.d.	7.9 n=1	7.6 ± 0.1 n=2	-
Pentachloroethane	48 hr	44.5	42.0	n.d.	7.2 ± 0.1 n=3	7.0 ± 0.0 n=4	7.2 ± 0.2 n=4
Hexachloroethane	48 hr	45.0	42.0	n.d.	7.4 ± 0.1 n=4	7.0 ± 0.1 n=4	7.6 ± 0.1 n=4
Tetrachloroethylene	48 hr	44.0	42.0	n.d.	n.d.	n.d.	n.d.
Tetrachloroethylene	28 day	44.5 ± 0.7 n=2	42.0 ± 0.0 n=2	n.d.	7.4 ± 0.2 n=32	7.0 ± 0.2 n=34	n.d.
1,3-Dichlorobenzene	48 hr	43.5	41.0	n.d.	n.d.	n.d.	n.d.
1,3-Dichlorobenzene	28 day	45.5 ± 1.8 n=3	42.2 ± 0.8 n=3	n.d.	7.4 ± 0.2 n=7	6.9 ± 0.1 n=8	-
1,2,4-Trichlorobenzene	48 hr	45.0	44.5	n.d.	n.d.	n.d.	n.d.

TABLE A-2 Cont. SUMMARY OF CONDITIONS AND WATER CHARACTERISTICS FOR MEASURED TOXICITY TESTS WITH DAPHNIA MAGNA IN LAKE SUPERIOR WATER

Compound	Test Duration	Hardness[a]	Alkalinity[a] $\bar{x}$ ± s.d.	Acidity[a] $\bar{x}$ ± s.d.	pH of New Solution $\bar{x}$ ± s.d. Fed or Unfed	pH of Old Solution $\bar{x}$ ± s.d.	
						Fed	Unfed
1,2,4-Trichlorobenzene	28 day	44.8 ± 0.6 n=4	41.9 ± 0.9 n=4	n.d.	7.3 ± 0.1 n=7	6.6 ± 0.1 n=8	-
Di-*n*-butylphthalate	48 hr	45.4[b] n=1	46.5[b] n=1	5.1[b] n=1	n.d.	-	7.1[b]
Chlordane	48 hr	42.2[b] n=1	58.0[b] n=1	2.5[b] n=1	n.d.	-	7.5[b] n=1
Arsenic^{+3}	28 day	47.2 ± 0.4 n=4	45.5 ± 1.3 n=4	-	n.d.	7.4 ± 0.2 n=73	-
Arsenic^{+3} [c]	48 hr	48.7 ± 2.0 n=16	46.8 ± 2.4 n=16	n.d.	8.1 ± 0.3 n=8	8.1 ± 0.4 n=4	7.9 ± 0.2 n=4
Nickel^{+2}	48 hr	51.1 ± 1.0 n=2	53.4 ± 2.8 n=4	n.d.	n.d.	-	8.8 ± 0.1 n=4

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[a] Values expressed as $mg \cdot L^{-1}$ of $CaCO_3$.

[b] Analysis performed on single pooled sample from selected exposure concentrations.

[c] Test conducted in dechlorinated city water from the City of Superior, Wisconsin.

APPENDIX B

TOXICITY TEST CHEMICAL CONCENTRATIONS

TABLE B-1. MEASURED CONCENTRATIONS OF TOXICANTS IN EXPOSURE WATER CHAMBERS (Mean ± S.D.)

			Concentrations ($\text{mg}\cdot\text{L}^{-1}$)			
Chemical	Organism	Duration	Control	A	B	C
1,2-Dichloroethane	*Daphnia magna*	48 h (Unfed)	0.0 ± 0.0 (n=2)	70.4 ± 2.8 (n=2)	99.2 ± 5.3 (n=2)	137 ± 8.5 (n=2)
1,2-Dichloroethane	" "	48 h (Fed)	0.0 ± 0.0 (n=2)	72.4 (n=1)	94.8 ± 11.6 (n=2)	143 (n=1)
1,2-Dichloroethane	" "	28 d	0.0 ± 0.0 (n=22)	10.6 ± 0.8 (n=16)	20.7 ± 1.7 (n=15)	41.6 ± 2.4 (n=14)
1,1,2-Trichloroethane	" "	48 h (Unfed)	0.0 ± 0.0 (n=2)	24.3 ± 1.8 (n=2)	40.1 ± 2.6 (n=2)	57.5 ± 2.9 (n=2)
1,1,2-Trichloroethane	" "	48 h (Fed)	0.0 ± 0.0 (n=2)	23 ± 3.5 (n=2)	40.1 ± 2.5 (n=2)	56.6 ± 4.2 (n=2)
1,1,2-Trichloroethane	" "	28 d	0.0 ± 0.0 (n=34)	1.72 ± 0.16 (n=21)	3.40 ± 0.29 (n=20)	6.35 ± 0.52 (n=20)
1,1,2,2-Tetrachloroethane	" "	48 h (Unfed)	0.0 ± 0.0 (n=2)	16.4 ± 1.3 (n=2)	22.8 (n=1)	33.3 ± 3.7 (n=2)
1,1,2,2-Tetrachloroethane	" "	48 h (Fed)	0.0 ± 0.0 (n=2)	16.3 ± 1.4 (n=2)	23.1 (n=1)	34.5 ± 2.1 (n=2)
1,1,2,2-Tetrachloroethane	" "	28 d	0.0 ± 0.0 (n=48)	0.419 ± 0.036 (n=24)	0.859 ± 0.085 (n=24)	1.71 ± 0.17 (n=24)
Pentachloroethane	" "	48 h (Unfed)	0.0 ± 0.0 (n=2)	1.7 ± 1.7 (n=2)	3.4 ± 2.8 (n=2)	5.2 ± 3.7 (n=2)
Pentachloroethane	" "	48 h (Fed)	0.0 ± 0.0 (n=2)	2.0 ± 1.3 (n=2)	3.83 ± 2.1 (n=2)	5.91 ± 2.7 (n=2)
Hexachloroethane	" "	48 h (Unfed)	0.0 ± 0.0 (n=2)	0.805 ± 0.14 (n=2)	1.08 ± 0.16 (n=2)	1.54 ± 0.08 (n=2)
Hexachloroethane	" "	48 h (Fed)	0.0 ± 0.0 (n=2)	0.631 ± 0.39 (n=2)	0.844 ± 0.49 (n=2)	1.12 ± 0.67 (n=2)
Hexachloroethane	*Salmo gairdneri*	192 h	0.00 ± 0.00 (n=10)	0.35 ± 0.06 (n=7)	0.64 ± 0.10 (n=7)	0.92 ± 0.05 (n=7)
Hexachloroethane	*Tanytarsus dissimilis*	72 h	0.004 ± 0.005 (n=4)	0.248 ± 0.038 (n=8)	0.619 ± 0.080 (n=8)	1.26 ± 0.14 (n=8)

TABLE B-1 Cont. MEASURED CONCENTRATIONS OF TOXICANTS IN EXPOSURE WATER CHAMBERS (Mean ± S.D.)

Chemical	Organism	Duration	Concentrations (mg·L^{-1}) D	 E	 F
1,2-Dichloroethane	*Daphnia magna*	48 h (Unfed)	188 ± 9.2 (n=2)	258 ± 17.7 (n=2)	405 ± 43.8 (n=2)
1,2-Dichloroethane	" "	48 h (Fed)	175 ± 27.6 (n=2)	244 ± 36.8 (n=2)	390 ± 65 (n=2)
1,2-Dichloroethane	" "	28 d	71.7 ± 4.8 (n=16)	94.4 ± 5.5 (n=13)	137 ± 9 (n=17)
1,1,2-Trichloroethane	" "	48 h (Unfed)	113 ± 7.1 (n=2)	159 ± 4.9 (n=2)	258 ± 9.1 (n=2)
1,1,2-Trichloroethane	" "	48 h (Fed)	107 ± 15.2 (n=2)	149 ± 18.4 (n=2)	241 ± 32.5 (n=2)
1,1,2-Trichloroethane	" "	28 d	13.2 ± 1.7 (n=18)	26.0 ± 2.2 (n=19)	41.8 ± 3.0 (n=19)
1,1,2,2-Tetrachloroethane	" "	48 h (Unfed)	46.9 ± 4.2 (n=2)	64.7 ± 4.6 (n=2)	94.9 ± 8.7 (n=2)
1,1,2,2-Tetrachloroethane	" "	48 h (Fed)	46.8 ± 4.5 (n=2)	63.8 ± 5.9 (n=2)	93.1 ± 11.1 (n=2)
1,1,2,2-Tetrachloroethane	" "	28 d	3.43 ± 0.39 (n=24)	6.85 ± 0.90 (n=23)	14.4 ± 1.4 (n=8)
Pentachloroethane	" "	48 h (Unfed)	9.2 ± 5.9 (n=2)	17 ± 8.2 (n=2)	30 ± 10 (n=2)
Pentachloroethane	" "	48 h (Fed)	9.53 ± 5.5 (n=2)	16.4 ± 9.1 (n=2)	28.5 ± 12.4 (n=2)
Hexachloroethane	" "	48 h (Unfed)	2.55 ± 0.35 (n=2)	4.65 ± 0.42 (n=2)	5.44 ± 0.63 (n=2)
Hexachloroethane	" "	48 h (Fed)	2.04 ± 1.08 (n=2)	3.81 ± 1.62 (n=2)	3.87 ± 2.84 (n=2)
Hexachloroethane	*Salmo gairdneri*	192 h	1.58 ± 0.19 (n=6)	1.83 ± 0.35 (n=3)	-
Hexachloroethane	*Tanytarsus dissimilis*	72 hr	2.75 ± 0.48 (n=8)	6.16 ± 0.54 (n=8)	-

TABLE B-1 Cont. MEASURED CONCENTRATIONS OF TOXICANTS IN EXPOSURE WATER CHAMBERS (Mean ± S.D.)

Chemical	Organism	Duration	Control	Concentrations ($mg \cdot L^{-1}$) A	 B	 C
Tetrachloroethylene	*Daphnia magna*	48 h (Unfed)	0.0 ± 0.0 (n=2)	2.56 ± 0.68 (n=2)	3.61 ± 1.33 (n=2)	7.00 ± 1.61 (n=2)
Tetrachloroethylene	" "	48 h (Fed)	0.0 ± 0.0 (n=2)	3.04 (n=1)	4.55 (n=1)	5.88 ± 3.20 (n=2)
Tetrachloroethylene	" "	28 d	0.0009 ± 0.0026 (n=20)	0.0759 ± 0.036 (n=10)	0.159 ± 0.085 (n=12)	0.254 ± 0.094 (n=8)
Tetrachloroethylene	*Tanytarsus dissimilis*	48 h	0.00 ± 0.00 (n=3)	4.38 ± 0.63 (n=6)	9.91 ± 0.48 (n=6)	21.5 ± 1.5 (n=6)
Tetrachloroethylene	*Salmo gairdneri*	96 h	0.00 ± 0.00 (n=5)	2.41 ± 0.22 (n=7)	3.69 ± 0.20 (n=7)	6.39 ± 0.65 (n=6)
Tetrachloroethylene/DMF	" "	96 h	0.00 ± 0.00/ 0.0 ± 0.0 (n=3/5)	2.23 ± 0.46/ 75.8 ± 13.9 (n=7/6)	3.53 ± 0.84/ 122 ± 12 (n=7/6)	5.95 ± 1.41/ 220 ± 11 (n=7/6)
1,2-Dichlorobenzene	" "	96 h	0.00 ± 0.00 (n=10)	0.75 ± 0.04 (n=7)	1.29 ± 0.07 (n=7)	2.05 ± 0.17 (n=7)
1,2-Dichlorobenzene	*Tanytarsus dissimilis*	48 h	0.00 ± 0.00 (n=3)	2.02 ± 0.49 (n=6)	5.53 ± 1.55 (n=6)	13.2 ± 2.2 (n=6)
1,3-Dichlorobenzene	*Daphnia magna*	48 h (Unfed)	0.0 ± 0.0 (n=2)	1.27 ± 0.05 (n=2)	2.14 ± 0.04 (n=2)	3.28 ± 0.21 (n=2)
1,3-Dichlorobenzene	" "	48 h (Fed)	0.0 ± 0.0 (n=2)	1.30 (n=1)	2.11 (n=1)	2.93 ± 0.70 (n=2)
1,3-Dichlorobenzene	" "	28 d	0.001 ± 0.004 (n=37)	0.044 ± 0.012 (n=21)	0.102 ± 0.023 (n=21)	0.182 ± 0.039 (n=20)
1,2,4-Trichlorobenzene	" "	48 h (Fed)	0.001 ± 0.001 (n=2)	0.293 ± 0.031 (n=2)	0.45 ± 0.017 (n=2)	0.850 ± 0.056 (n=2)
1,2,4-Trichlorobenzene	" "	48 h (Unfed)	0.001 ± 0.001 (n=2)	0.276 ± 0.055 (n=2)	0.428 ± 0.049 (n=2)	0.762 ± 0.18 (n=2)
1,2,4-Trichlorobenzene	" "	28 d	0.000 ± 0.001 (n=31)	0.018 ± 0.003 (n=20)	0.039 ± 0.005 (n=19)	0.079 ± 0.011 (n=20)
1,2,4-Trichlorobenzene	*Salmo gairdneri*	192 h	0.00 ± 0.00 (n=9)	0.44 ± 0.03 (n=12)	0.57 ± 0.05 (n=12)	1.09 ± 0.05 (n=12)

TABLE B-1 Cont. MEASURED CONCENTRATIONS OF TOXICANTS IN EXPOSURE WATER CHAMBERS (Mean ± S.D.)

			Concentrations ($mg \cdot L^{-1}$)		
Chemical	Organism	Duration	D	E	F
Tetrachloroethylene	Daphnia magna	48 h (Unfed)	11.5 ± 2.30 (n=2)	19.1 ± 2.33 (n=2)	31.0 ± 3.04 (n=2)
Tetrachloroethylene	" "	48 h (Fed)	9.61 ± 4.94 (n=2)	15.6 ± 7.28 (n=2)	25.6 ± 10.6 (n=2)
Tetrachloroethylene	" "	28 d	0.505 ± 0.250 (n=10)	1.11 ± 0.48 (n=8)	1.75 ± 1.10 (n=6)
Tetrachloroethylene	Tanytarsus dissimilis	48 h	47.2 ± 6.0 (n=6)	101.8 ± 0.8 (n=2)	-
Tetrachloroethylene	Salmo gairdneri	96 h	11.2 ± 0.2 (n=2)	17.3 ± 1.0 (n=2)	-
Tetrachloroethylene/DMF	" "	96 h	11.3 ± 0.9 326 ± 16 (n=3/2)	16.4 ± 1.5/ 513 ± 17 (n=3/2)	-
1,2-Dichlorobenzene	" "	96 h	3.07 ± 0.03 (n=2)	3.81 ± 0.43 (n=3)	-
1,2-Dichlorobenzene	Tanytarsus dissimilis	48 h	21.9 ± 7.4 (n=5)	45.8 ± 16.1 (n=3)	-
1,3-Dichlorobenzene	Daphnia magna	48 h (Unfed)	5.89 ± 0.89 (n=2)	10.2 ± 1.52 (n=2)	17.2 ± 2.05 (n=2)
1,3-Dichlorobenzene	" "	48 h (Fed)	4.85 ± 2.36 (n=2)	9.53 ± 2.50 (n=2)	16.0 ± 3.68 (n=2)
1,3-Dichlorobenzene	" "	28 d	0.373 ± 0.053 (n=21)	0.689 ± 0.156 (n=20)	1.45 ± 0.28 (n=21)
1,2,4-Trichlorobenzene	" "	48 h (Fed)	1.32 ± 0.057 (n=2)	1.68 ± 0.092 (n=2)	2.64 ± 0.16 (n=2)
1,2,4-Trichlorobenzene	" "	48 h (Unfed)	1.21 ± 0.21 (n=2)	1.61 ± 0.18 (n=2)	2.63 ± 0.17 (n=2)
1,2,4-Trichlorobenzene	" "	28 d	0.162 ± 0.028 (n=19)	0.363 ± 0.056 (n=20)	0.694 ± 0.140 (n=19)
1,2,4-Trichlorobenzene	Salmo gairdneri	192 h	1.70 ± 0.12 (n=12)	2.82 ± 0.17 (n=4)	-

TABLE B-1 Cont. MEASURED CONCENTRATIONS OF TOXICANTS IN EXPOSURE WATER CHAMBERS (Mean ± S.D.)

Chemical	Organism	Duration	Concentrations ($mg \cdot L^{-1}$) Control	A	B	C
Pentachlorobenzene/DMF	*Salmo gairdneri*	144 h	0.00 ± 0.00/ 437 ± 136 (n=14/4)	0.06 ± 0.02/ 365 ± 193 (n=14/4)	0.12 ± 0.03/ 408 ± 138 (n=14/4)	0.28 ± 0.08/ 380 ± 172 (n=14/4)
Hexachlorobenzene/DMF	" "	96 h	0.00014 ± 0.00016/ 922 ± 114 (n=9/3)	0.0038 ± 0.0003/ 947 ± 40 (n=10/3)	-	-
Hexachlorobenzene/DMF	*Lepomis macrochirus*	96 h	0.0000 ± 0.0000/ 943 ± 106 (n=10/6)	0.0041 ± 0.0003/ 878 ± 144 (n=10/6)	-	-
Hexachlorobenzene/DMF	*Tanytarsus dissimilis*	48 h	0.0000 ± 0.0000/ 0 (n=4/1)	0.0000 ± 0.0000/ 1391 (n=4/1)	-	0.0028 ± 0.0009/ 1018 (n=4/1)
Hexachlorobutadiene	*Salmo gairdneri*	96 h	0.00 ± 0.00 (n=10)	0.067 ± 0.01 (n=7)	0.096 ± 0.01 (n=7)	0.229 ± 0.02 (n=7)
Hexachlorobutadiene	*Lepomis macrochirus*	96 h	0.0000 ± 0.0000 (n=12)	0.0462 ± 0.0064 (n=12)	0.0846 ± 0.0137 (n=12)	0.126 ± 0.016 (n=12)
Di-*n*-butylphthalate	*Daphnia magna*	48 h (Unfed)	< 0.32 ± 0.13 (n=4)	0.54 ± 0.14 (n=12)	1.16 ± 0.31 (n=12)	2.51 ± 0.40 (n=12)
Pentachlorophenol	*Gammarus pseudolimnaeus*	96 h	0.000 ± 0.000 (n=7)	0.108 ± 0.004 (n=7)	0.184 ± 0.008 (n=7)	0.287 ± 0.023 (n=7)
Pentachlorophenol	*Tanytarsus dissimilis*	48 h	< 0.05 ± 0.00 (n=4)	11.6 ± 0.6 (n=4)	18.0 ± 6.5 (n=4)	46.4 ± 6.4 (n=4)
Heptachlor[a/]	*Selenastrum capricornutum* (Test 1)	96 h	< 0.0008 (n=1)	0.0086 (n=1)	0.0176 (n=1)	0.0284 (n=1)
Heptachlor[a/]	*Selenastrum capricornutum* (Test 2)	96 h	< 0.0008 (n=1)	0.0136 (n=1)	0.0228 (n=1)	0.0444 (n=1)
Chlordane	*Daphnia magna*	48 h (Unfed)	0.0000 ± 0.0000 (n=6)	0.0118 ± 0.0019 (n=5)	0.0227 ± 0.0018 (n=6)	0.0463 ± 0.0039 (n=6)
Toxaphene[a/]	*Selenastrum capricornutum*	96 h	0.0 (n=1)	0.25 (n=1)	0.48 (n=1)	0.85 (n=1)

TABLE B-1 Cont. MEASURED CONCENTRATION OF TOXICANTS IN EXPOSURE WATER CHAMBERS (Mean ± S.D.)

Chemical	Organism	Duration	Concentrations (mg·L^{-1})		
			D	E	F
Pentachlorobenzene/DMF	*Salmo gairdneri*	144 h	0.44 ± 0.09/ 402 ± 146 (n=14/4)	0.71 ± 0.09/ 372 ± 133 (n=14/4)	-
Hexachlorobenzene/DMF	" "	96 h	-	0.0809 ± 0.0078/ 929 ± 53 (n=9/3)	-
Hexachlorobenzene/DMF	*Lepomis macrochirus*	96 h	-	0.0774 ± 0.0057/ 862 ± 147 (n=10/6)	-
Hexachlorobenzene/DMF	*Tanytarsus dissimilis*	48 h	-	0.0581 ± 0.0169/ 850 (n=4/1)	-
Hexachlorobutadiene	*Salmo gairdneri*	96 h	0.468 ± 0.08 (n=7)	0.670 ± 0.10 (n=7)	-
Hexachlorobutadiene	*Lepomis macrochirus*	96 h	0.228 ± 0.025 (n=12)	0.445 ± 0.036 (n=7)	-
Di-*n*-butylphthalate	*Daphnia magna*	48 h (Unfed)	5.45 ± 0.98 (n=9)	11.2 ± 2.32 (n=8)	-
Pentachlorophenol	*Gammarus pseudolimnaeus*	96 h	0.462 ± 0.036 (n=7)	0.761 ± 0.141 (n=4)	-
Pentachlorophenol	*Tanytarsus dissimilis*	48 h	83.4 ± 13.2 (n=4)	197 ± 9.3 (n=4)	-
Heptachlor[a]	*Selenastrum capricornutum* (Test 1)	96 h	0.0385 (n=1)	0.0444 (n=1)	-
Heptachlor[a]	*Selenastrum capricornutum* (Test 2)	96 h	0.0570 (n=1)	0.107 (n=1)	-
Chlordane	*Daphnia magna*	48 h (Unfed)	0.0871 ± 0.0076 (n=6)	0.1733 ± 0.0118 (n=6)	-
Toxaphene[a]	*Selenastrum capricornutum*	96 h	0.87 (n=1)	1.93 (n=1)	-

TABLE B-1 Cont. MEASURED CONCENTRATIONS OF TOXICANTS IN EXPOSURE WATER CHAMBERS (Mean ± S.D.)

Chemical	Organism	Duration	Control	Concentrations ($mg \cdot L^{-1}$) A	B	C
Dimethylformamide	Salmo gairdreri	96 h	<80 ± 27 (n=6)	1900 ± 100 (n=7)	2,800 ± 170 (n=7)	4,900 ± 240 (n=7)
Dimethylformamide	Lepomis macrochirus	96 h	0.0 ± 0.0 (n=8)	3,500 ± 790 (n=8)	5,200 ± 500 (n=8)	6,600 ± 260 (n=8)
Dimethylformamide	Pimephales promelas	96 h	3.1 ± 1.4 (n=7)	5,100 ± 360 (n=7)	6,400 ± 470 (n=7)	7,800 ± 150 (n=7)
Dimethylformamide	Tanytarsus dissimilis	48 h	0.0 ± 0.0 (n=4)	25,000 ± 3,800 (n=4)	33,000 ± 2,600 (n=4)	47,000 ± 1,700 (n=4)
Dimethylformamide	Daphnia magna	48 h (Unfed)	0.0 (n=2)	2,200 (n=2)	6,000 (n=2)	11,000 (n=2)
Methanol	Salmo gairdneri	96 h	0.0 ± 0.0 (n=8)	3,720 ± 560 (n=7)	6,400 ± 550 (n=7)	10,700 ± 520 (n=7)
Methanol	Lepomis macrochirus	96 h	0.0 ± 0.0 (n=8)	3,500 ± 140 (n=7)	6,180 ± 140 (n=7)	10,850 ± 540 (n=7)
Methanol	Pimephales promelas	96 h	0.0 ± 0.0 (n=10)	3,900 ± 500 (n=10)	7,500 ± 1,800 (n=10)	13,400 ± 2,000 (n=10)
Arsenic^{+3}	Jordanella floridae	96 h	0.00 ± 0.00 (n=7)	5.06 ± 1.19 (n=8)	13.13 ± 1.11 (n=7)	25.91 ± 3.49 (n=7)
Arsenic^{+3}	Jordanella floridae	30 d (Test 1)	0.00 ± 0.00 (n=24)	0.30 ± 0.12 (n=24)	0.60 ± 0.24 (n=24)	1.20 ± 0.43 (n=24)
Arsenic^{+3}	Jordanella floridae	30 d (Test 2)	0.00 ± 0.00 (n=17)	1.24 ± 0.35 (n=20)	2.13 ± 0.38 (n=20)	4.12 ± 0.29 (n=20)
Arsenic^{+3}	Pimephales promelas	96 h	0.00 ± 0.00 (n=7)	5.06 ± 1.19 (n=8)	13.13 ± 1.11 (n=7)	25.91 ± 3.49 (n=7)
Arsenic^{+3}	" "	30 d	0.00 ± 0.00 (n=13)	1.06 ± 0.28 (n=18)	2.13 ± 0.39 (n=18)	4.30 ± 0.50 (n=18)
Arsenic^{+3}	Gammarus pseudolimnaeus	96 h	0.00 ± 0.00 (n=5)	0.30 ± 0.03 (n=6)	0.58 ± 0.07 (n=7)	1.34 ± 0.18 (n=6)
Arsenic^{+3}	Daphnia magna	48 h (Unfed)	< 0.002 ± 0.000 (n=6)	0.832 ± 0.039 (n=6)	1.11 ± 0.05 (n=6)	1.83 ± 0.08 (n=6)

TABLE B-1 Cont. MEASURED CONCENTRATION OF TOXICANTS IN EXPOSURE WATER CHAMBERS (Mean ± S.D.)

Chemical	Organism	Duration	Concentrations (mg·L^{-1}) D	 E	 F
Dimethylformamide	Salmo gairdneri	96 h	7,800 ± 370 (n=7)	15,000 ± 60 (n=3)	-
Dimethylformamide	Lepomis macrochirus	96 h	8,800 ± 470 (n=8)	9,400 ± 320 (n=8)	-
Dimethylformamide	Pimephales promelas	96 h	9,500 ± 440 (n=7)	12,000 ± 340 (n=7)	-
Dimethylformamide	Tanytarsus dissimilis	48 h	66,000 ± 2,500 (n=4)	93,000 ± 2,200 (n=4)	-
Dimethylformamide	Daphnia magna	48 h (Unfed)	22,000 (n=2)	41,000 (n=2)	-
Methanol	Salmo gairdneri	96 h	16,300 ± 420 (n=7)	25,800 ± 140 (n=2)	-
Methanol	Lepomis macrochirus	96 h	16,300 ± 450 (n=7)	28,590 ± 1,320 (n=2)	-
Methanol	Pimephales promelas	96 h	23,200 ± 2,000 (n=10)	36,200 ± 2,700 (n=4)	-
Arsenic^{+3}	Jordanella floridae	96 h	52.05 ± 1.26 (n=3)	99.69 ± 10.18 (n=3)	-
Arsenic^{+3}	Jordanella floridae	30 d (Test 1)	2.34 ± 0.81 (n=24)	5.04 ± 1.82 (n=24)	-
Arsenic^{+3}	" "	30 d (Test 2)	7.57 ± 0.61 (n=20)	16.32 ± 0.85 (n=20)	-
Arsenic^{+3}	Pimephales promelas	96 h	52.05 ± 1.26 (n=3)	99.69 ± 10.18 (n=3)	-
Arsenic^{+3}	" "	30 d	7.37 ± 0.49 (n=18)	16.48 ± 1.03 (n=18)	-
Arsenic^{+3}	Gammarus pseudolimnaeus	96 h	2.40 ± 0.24 (n=5)	5.25 ± 1.63 (n=4)	-
Arsenic^{+3}	Daphnia magna	48 h (Unfed)	2.55 ± 0.23 (n=5)	4.19 ± 0.16 (n=4)	6.46 ± 0.92 (n=4)

TABLE B-1 Cont. MEASURED CONCENTRATIONS OF TOXICANTS IN EXPOSURE WATER CHAMBERS (Mean ± S.D.)

Chemical	Organism	Duration	Concentrations ($mg \cdot L^{-1}$) Control	A	B	C
Arsenic^{+3}	Daphnia magna	48 h (Fed)	<0.002 ± 0.000 (n=4)	1.04 ± 0.11 (n=6)	1.54 ± 0.10 (n=4)	2.21 ± 0.03 (n=6)
Arsenic^{+3}	" "	28 d	<0.002 ± 0.001 (n=12)	0.073 ± 0.006 (n=12)	0.13 ± 0.005 (n=12)	0.27 ± 0.014 (n=12)
Chromium^{+6}	Gammarus pseudolimnaeus	96 h	0.0008 ± 0.0008 (n=5)	0.0290 ± 0.0028 (n=7)	0.0635 ± 0.0057 (n=7)	0.137 ± 0.0073 (n=7)
Chromium^{+6}	Tanytarsus dissimilis	48 h	1.00 ± 0.00 (n=4)	26.0 ± 0.4 (n=4)	51.0 ± 1.6 (n=4)	104 ± 2 (n=4)
Lead^{+2}	Gammarus pseudolimnaeus	96 h	0.0018 ± 0.0010 (n=4)	0.047 ± 0.009 (n=6)	0.097 ± 0.010 (n=4)	0.202 ± 0.019 (n=4)
Lead^{+2}	Tanytarsus dissimilis	48 h	0.0 ± 0.0 (n=2)	8.7 ± 5.9 (n=4)	40 ± 9.2 (n=4)	210 ± 14 (n=4)
Mercury^{+2}	Pimephales promelas	96 h	0.0000 ± 0.0000 (n=7)	0.0630 ± 0.0086 (n=7)	0.134 ± 0.0119 (n=7)	0.207 ± 0.028 (n=7)
Mercury^{+2}	" "	35 d	0.00001 ± 0.00001 (n=22)	0.00023 ± 0.00003 (n=22)	0.00048 ± 0.00007 (n=22)	0.00087 ± 0.00008 (n=22)
Nickel^{+2}	Daphnia magna	48 h (Unfed)	0.030 ± 0.041 (n=2)	0.249 ± 0.031 (n=4)	0.453 ± 0.008 (n=4)	0.723 ± 0.221 (n=4)
Silver^{+1}	Pimephales promelas	96 h	0.00013 ± 0.00017 (n=5)	0.00334 ± 0.00102 (n=7)	0.00838 ± 0.00492 (n=7)	0.01374 ± 0.00376 (n=7)
Silver^{+1}	Jordanella floridae	96 h	0.00013 ± 0.00017 (n=5)	0.00334 ± 0.00102 (n=7)	0.00838 ± 0.00492 (n=7)	0.01374 ± 0.00376 (n=7)
Silver^{+1}	Tanytarsus dissimilis	48 h	0.000 ± 0.000 (n=6)	0.371 ± 0.054 (n=6)	0.842 ± 0.047 (n=6)	1.870 ± 0.130 (n=6)
Silver^{+1}	Gammarus pseudolimnaeus	96 h	0.00017 ± 0.00005 (n=6)	0.00080 ± 0.00017 (n=5)	0.00215 ± 0.00065 (n=8)	0.00488 ± 0.00056 (n=7)
Selenium^{+4}	Tanytarsus dissimilis	48 h	0.0 ± 0.0 (n=2)	19.8 ± 0.4 (n=4)	39.7 ± 0.4 (n=4)	80.9 ± 0.4 (n=2)

TABLE B-1 Cont. MEASURED CONCENTRATION OF TOXICANTS IN EXPOSURE WATER CHAMBERS (Mean ± S.D.)

Chemical	Organism	Duration	Concentrations ($mg \cdot L^{-1}$) D	E	F
Arsenic^{+3}	*Daphnia magna*	48 h (Fed)	4.22 ± 0.53 (n=6)	7.85 ± 0.21 (n=4)	13.3 ± 0.52 (n=4)
Arsenic^{+3}	" "	28 d	0.63 ± 0.034 (n=11)	1.32 ± 0.032 (n=12)	2.68 ± 0.059 (n=11)
Chromium^{+6}	*Gammarus pseudolimnaeus*	96 h	0.280 ± 0.014 (n=7)	0.548 ± 0.013 (n=4)	-
Chromium^{+6}	*Tanytarsus dissimilis*	48 h	205 ± 6 (n=4)	412 ± 11 (n=4)	-
Lead^{+2}	*Gammarus pseudolimnaeus*	96 h	0.420 ± 0.036 (n=4)	0.878 ± 0.029 (n=4)	-
Lead^{+2}	*Tanytarsus dissimilis*	48 h	650 ± 29 (n=4)	1800 ± 95 (n=4)	-
Mercury^{+2}	*Pimephales promelas*	96 h	0.482 ± 0.024 (n=4)	- b/	-
Mercury^{+2}	" "	35 d	0.0018 ± 0.0002 (n=22)	0.0037 ± 0.0006 (n=21)	-
Nickel^{+2}	*Daphnia magna*	48 h (Unfed)	1.77 ± 0.019 (n=4)	3.77 ± 0.032 (n=4)	-
Silver^{+1}	*Pimephales promelas*	96 h	0.02955 ± 0.00662 (n=7)	0.06791 ± 0.01496 (n=3)	-
Silver^{+1}	*Jordanella floridae*	96 h	0.02955 ± 0.00662 (n=7)	0.06791 ± 0.01496 (n=3)	-
Silver^{+1}	*Tanytarsus dissimilis*	49 h	3.350 ± 0.064 (n=6)	7.190 ± 0.290 (n=6)	-
Silver^{+1}	*Gammarus pseudolimnaeus*	96 h	0.01527 ± 0.00385 (n=3)	0.03561 ± 0.00800 (n=3)	-
Selenium^{+4}	*Tanytarsus dissimilis*	48 h	164 ± 2 (n=2)	325 ± 10 (n=2)	-

TABLE B-1 Cont. MEASURED CONCENTRATIONS OF TOXICANTS IN EXPOSURE WATER CHAMBERS (Mean ± S.D.)

Chemical	Organism	Duration	Concentrations ($mg \cdot L^{-1}$) Control	A	B	C
Cyanide (as HCN)	*Tanytarsus dissimilis*	48 h	0.00 ± 0.00 (n=2)	0.86 ± 0.01 (n=2)	1.66 ± 0.01 (n=2)	3.27 ± 0.06 (n=2)
Cyanide (as CN^-)	" "	48 h	0.00 ± 0.00 (n=3)	0.88 ± 0.02 (n=6)	1.72 ± 0.11 (n=6)	3.50 ± 0.18 (n=6)

TABLE B-1 Cont. MEASURED CONCENTRATION OF TOXICANTS IN EXPOSURE WATER CHAMBERS (Mean ± S.D.)

Chemical	Organism	Duration	Concentrations ($mg \cdot L^{-1}$) D	E	F
Cyanide (as HCN)	_Tanytarsus dissimilis_	48 h	5.31 ± 0.25 (n=2)	7.14 ± 0.23 (n=2)	-
Cyanide (as CN^-)	" "	48 h	6.56 ± 1.16 (n=6)	13.1 ± 0.4 [c/] (n=2)	-

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a/ Initial concentrations of heptachlor or toxaphene.

b/ Due to analytical problems and high variability of concentrations, this exposure was not used in determination of the LC_{50} concentration.

c/ Due to high pH and resultant effect upon the equilibrium between HCN and CN^-, this concentration was not used in determination of the LC_{50} concentration.

APPENDIX C

PURITY LEVELS, ANALYTICAL PARAMETERS AND PROCEDURES, AND ANALYTICAL QUALITY CONTROL DATA FOR TOXICITY TEST CHEMICALS

TABLE C-1. SOURCES AND PURITY LEVELS OF CHEMICALS USED IN TOXICITY TESTS

Chemical	Source	Lot No.	Purity
1,2-Dichloroethane	Aldrich Chem. Co.	-	99%
1,1,2-Trichloroethane	Aldrich Chem. Co.	JB070177/HD061197	95%
1,1,2,2-Tetrachloroethane	Aldrich Chem. Co.	LB090677	98%
Pentachloroethane	Aldrich Chem. Co.	CD072484	96%
Hexachloroethane	Aldrich Chem. Co.	ED080787	98%
Tetrachloroethylene	Aldrich Chem. Co.	JB072677	99%
1,2-Dichlorobenzene	Aldrich Chem. Co.	-	99%
1,3-Dichlorobenzene	Aldrich Chem. Co.	PC110987	98%
1,4-Dichlorobenzene	Aldrich Chem. Co.	-	97%
1,2,4-Trichlorobenzene	Aldrich Chem. Co.	073034	99%
Pentachlorobenzene	Aldrich Chem. Co.	-	98%
Hexachlorobenzene	Aldrich Chem. Co.	JC010487	97%
Hexachlorobutadiene	Aldrich Chem. Co.	PB082017	98%
Di-*n*-butylphthalate	Monsanto Co.	-	99.8%
Pentachlorophenol	Aldrich Chem. Co.	P260-4	99^+%
Heptachlor	EPA/RTP	-	98%
Chlordane	EPA/FDA	20(69-18)	Technical grade
Toxaphene	Hercules	X-16189-49	Technical grade
Dimethylformamide	Burdick & Jackson	AD665	98%
Methanol	Burdick & Jackson	AB468/AE562	99^+%
As^{+3} ($NaAsO_2$)	Fisher	776308	ACS Grade
Cr^{+6} ($K_2Cr_2O_7$)	Fisher	787060	99.96%
Pb^{+2} [$Pb(NO_3)_2$]	Fisher	791928	ACS Grade
Hg^{+2} ($HgCl_2$)	Ventron (Alpha)	021971	ACS Grade
Ni^{+2} [$Ni(NO_3)_2$] · 6 H_2O	Mallinckrodt	6376	99.9%
Ag^{+1} ($AgNO_3$)	Fisher	732462	99.994%
Se^{+4} (SeO_2)	Fisher	764220	95.2%
CN^{-1} (NaCN)	Fisher	702579	98.8%

TABLE C-2. SUMMARY OF GAS-LIQUID CHROMATOGRAPH PARAMETERS FOR ORGANIC TEST CHEMICALS

Compound	Instrument[a/]	Column Packing	Isothermal Column Temp (°C)	Retention Time (Min)
1,2-Dichloroethane	Hewlett-Packard 5710A	4% SE-30/6% OV-210	50	3.03
1,1,2-Trichloroethane	Hewlett-Packard 5710A	4% SE-30/6% OV-210	50	2.68
1,1,2,2-Tetrachloroethane	Hewlett-Packard 5710A	1.5% OV-17/1.95% QF-1	75	1.68
Pentachloroethane	Hewlett-Packard 5710A	1.5% OV-17/1.95% QF-1	90	1.80
Hexachloroethane	Tracor 550	3% OV-101	130	1.30
Tetrachloroethylene	Tracor 550	3% OV-101	65	1.60
1,2-Dichlorobenzene	Tracor 550	3% OV-101	120	1.44
1,3-Dichlorobenzene	Hewlett-Packard 5710A	4% SE-30/6% OV-210	100	2.36
1,4-Dichlorobenzene	Tracor 550	3% OV-101	120	1.35
1,2,4-Trichlorobenzene	Tracor 550	3% OV-101	120	2.48
1,2,4-Trichlorobenzene	Hewlett-Packard 5710A	1.5% OV-17/1.95% QF-1	110	2.29
1,2,4-Trichlorobenzene	Hewlett-Packard 5710A	4% SE-30/6% OV-210	120	2.94
Pentachlorobenzene	Tracor MT 160	3% OV-101	205	1.18
Hexachlorobenzene	Tracor MT 160	3% OV-101	205	2.41
Hexachlorobutadiene	Tracor 550	3% OV-101	120	2.35
Di-_n_-butylphthalate	Tracor MT-160	3% OV-101	225	2.7
Pentachlorophenol	Hewlett-Packard 5730A	1.5% SP2250/1.95%SP2401	160	5.2
Heptachlor	Tracor MT-220	4% SE-30/6% OV-210	185	0.90
Chlordane	Tracor 550	3% OV-101	205	7.57, 8.25[b/]
Toxaphene	Tracor MT-220	4% SE-30/6% OV-210	185	-[c/]
Methanol	Hewlett-Packard 5730A	Tenax GC®	80	1.1
Dimethylformamide	Hewlett-Packard 5730A	Tenax GC®	160	1.6

a/ ^{63}Ni electron-capture detectors used for analysis of compounds, except for methanol and dimethylformamide where flame-ionization detectors were used.

b/ Retention times for _trans_- and _cis_- chlordane isomers, respectively.

c/ Multiple compounds present in toxaphene mixture eluted over a range of retention times.

TABLE C-3. SUMMARY OF EXTRACTION PROCEDURES AND PERCENTAGE RECOVERIES FOR ORGANIC TEST CHEMICALS ANALYZED BY GAS-LIQUID CHROMATOGRAPHY

Compound	Volume of Water Sample (mL)	Extraction Solvent (Volume Used)	Extraction Apparatus	% Recovery of Spiked Samples $\bar{x} \pm$ s.d.
1,2-Dichloroethane	75	Isooctane (25 mL)	100 mL vol. flask on magnetic stirrer	103.1 ± 5.6 (n=18)
1,1,2-Trichloroethane	75	Isooctane (25 mL)	100 mL vol. flask on magnetic stirrer	96.6 ± 7.0 (n=15)
1,1,2,2-Tetrachloroethane	75	Isooctane (50 mL)	200 mL vol. flask on magnetic stirrer	100.2 ± 8.2 (n=8)
Pentachloroethane	75	Isooctane (25 mL)	100 mL vol. flask on magnetic stirrer	98 (n=1)
Hexachloroethane	5-50	Hexane (50 mL)	100 mL vol. flask on magnetic stirrer	96.7 ± 2.9 (n=16)
Tetrachloroethylene	5-50	Hexane (50 mL)	100 mL vol. flask on magnetic stirrer	89.9 ± 6.2 (n=23)
1,2-Dichlorobenzene	2-50	Hexane (50 mL)	100 mL vol. flask on magnetic stirrer	103.7 ± 2.6 (n=15)
1,3-Dichlorobenzene	75	Isooctane (25 mL)	100 mL vol. flask on magnetic stirrer	95.4 ± 4.7 (n=15)
1,4-Dichlorobenzene	2-50	Hexane (50 mL)	100 mL vol. flask on magnetic stirrer	100.1 ± 3.0 (n=19)
1,2,4-Trichlorobenzene	2-50	Petroleum ether (50 mL)	100 mL vol. flask on magnetic stirrer	98.8 ± 2.3 (n=18)
1,2,4-Trichlorobenzene	75	Isooctane (25 mL)	100 mL vol. flask on magnetic stirrer	98.5 ± 8.4 (n=15)
Pentachlorobenzene	1-5	Hexane (5 mL)[a]	18 mL glass-stoppered test tube	94.3 ± 4.0 (n=21)
Hexachlorobenzene	1-5	Hexane (5 mL)[a]	18 mL glass-stoppered test tube	96.6 ± 3.1 (n=23)

TABLE C-3 Cont. SUMMARY OF EXTRACTION PROCEDURES AND PERCENTAGE RECOVERIES FOR ORGANIC TEST CHEMICALS ANALYZED BY GAS-LIQUID CHROMATOGRAPHY

Compound	Volume of Water Sample (mL)	Extraction Solvent (Volume Used)	Extraction Apparatus	% Recovery of Spiked Samples $\bar{x}$ ± s.d.
Hexachlorobutadiene	75	Isooctane (25 mL)	100 mL vol. flask on magnetic stirrer	100.7 ± 5.5 (n=16)
Di-n-butylphthalate	50	Hexane (50 mL)	100 mL vol. flask on magnetic stirrer	96.9 ± 9.2 (n=17)
Pentachlorophenol (Midge and *Gammarus*)	100	Hexane (100 mL)b/	200 mL vol. flask on magnetic stirrer	99.3 ± 4.6 (n=4) 97.2 ± 6.0 (n=6)
Heptachlor	10	Hexane (10 mL)	50 mL vol. flask on magnetic stirrer	100.7 ± 7.5 (n=3)
Chlordane	5-50	Hexane (50 mL)	100 mL vol. flask on magnetic stirrer	99.9 ± 7.6 (n=21)
Toxaphene	50	Hexane (50 mL)	100 mL vol. flask on magnetic stirrer	93.3 ± 6.9 (n=4)
Methanol	_c/	_c/	_c/	102.2 ± 3.1 (n=10)
Dimethylformamide	_d/	_d/	_d/	100.2 ± 0.4 (n=6)

a/ Three drops saturated NaCl solution added.

b/ Concentrated H_2SO_4 (2.0 mL) added prior to extraction, and extract derivatized with diazomethane.

c/ Analyzed by direct aqueous injection GLC technique for rainbow trout and bluegill sunfish acute tests.

d/ Analyzed by direct aqueous injection GLC technique for rainbow trout acute test.

TABLE C-4. SUMMARY OF ANALYTICAL TECHNIQUES AND QUALITY CONTROL PARAMETERS FOR TOXICITY TESTS WITH SELECTED ELEMENTS AND AQUATIC ORGANISMS

Element	Salt	Organism	Method	Atomization	% LSW[a] in Samples & Stds.	% Spike Recovery	% Agreement of Duplicates
Arsenic	$NaAsO_2$	Fathead minnow Flagfish	EPA[b] #206.2	Furnace	≤ 2%[c]	100.4 ± 6.1 (n=57)	96.2 ± 3.7 (n=25)
Chromium	$K_2Cr_2O_7$	Midge	EPA #218.1	Flame	25	106.0 (n=2)	99.6 (n=2)
Chromium	$K_2Cr_2O_7$	*Gammarus*	EPA #218.1	Furnace	25	100.5 ± 2.0 (n=4)	97.3 ± 3.5 (n=5)
Lead	$Pb(NO_3)_2$	Midge	EPA #239.1	Flame	50	99.7 (n=2)	98.4 (Filtered-0.45 μ) (n=2) 91.7 (Unfiltered) (n=2)
Lead	$Pb(NO_3)_2$	*Gammarus*	EPA #239.1	Furnace	25	n.d.[d]	93.4 ± 5.9 (n=5)
Nickel	$Ni(NO_3)_2 \cdot 6H_2O$	*Daphnia*	EPA #249.2	Furnace	50	100.8 ± 2.9 (n=6)	96.0 ± 4.2 (n=3)
Selenium	SeO_2	Midge	Perkin-Elmer (1976)[e]	Flame	99.5	96.0 (n=2)	96.8 (n=2)
Silver	$AgNO_3$	Fathead minnow Flagfish Midge *Gammarus*	EPA #272.2	Furnace	10-99.5	96.7 ± 9.0 (n=24)	95.9 ± 2.8 (n=12)
Mercury	$HgCl_2$	Fathead minnow	EPA #245.1 Modified	Cold vapor	_[f]	102.5 ± 7.1 (Acute test) (n=12) 95.7 ± 5.0 (Early life-stage test) (n=12)	_[g]

[a] Lake Superior water used in samples and standards to overcome any matrix interference.

[b] U.S. EPA. 1979. Methods for Chemical Analysis of Water and Waste. EPA 600-4-79-020.

[c] Controls contained 50% and all samples contained ≤2% of dechlorinated city water.

[d] Not determined because spiked samples and standards were prepared identically.

[e] Perkin-Elmer Corp. 1976. Analytical Methods for Atomic Absorption Spectrophotometry (Revised Sept., 1976). Perkin-Elmer Corp. Publ., Norwalk, Conn.

[f] Deionized water was used for standard preparation and sample dilution.

[g] The mean coefficient of variation from triplicate analyses of the same samples was 5.3% (n=8).